Peru PREVEN Study

URBAN COMMUNITY RANDOMIZED TRIAL FOR STD PREVENTION

Study Documents

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PROTOCOL SUMMARY - 2010

A 20 City Urban Community Randomized Trial of STD Prevention

I. Introduction

This document summarizes the protocol implemented in the Peruvian Urban Community Randomization Trial of STD Prevention (The Peru PREVEN Study) and includes protocol revisions over the course of this 10 year multi-component ("complex") intervention. When the proposal was originally submitted to the Wellcome Trust/Burroughs Wellcome Fund Infection Awareness Initiative in 1999 in collaboration with the National Peruvian STD Program, the study was designed as a three arm trial; the outcomes were the combined prevalences of sexually transmitted infections (STI) in women in antenatal clinics and military recruits, and in female sex workers (FSW). However, as is often the case for complex interventions[1-3], a variety of circumstances led to modification of the protocol.

Between proposal submission and study initiation, the overall funding from the Wellcome Trust was reduced by 25%, post-election political changes resulted in the deterioration of the Peruvian National STD/HIV Control Program, military service became no longer obligatory, and HIV surveillance in antenatal care was separated from the National STD/HIV Control Program and then weakened. PREVEN Study leaders in Peru transitioned from the National STD/HIV Control Program to a base at Universidad Peruana Cayetano Heredia (UPCH). The study protocol was therefore re-designed to include two, rather than three arms, with outcome measures planned for random samples of the general population of young adults and female sex workers, rather than samples of antenatal women and military recruits. The major protocol changes were communicated to our Wellcome Trust Programme Officer, Michael Chew, on March 14, 2002.

The outcome measures were determined *a priori*, as documented in our trial summary presented to the DSMB in April, 2006, which is attached. Following suggestions by the DSMB, the analysis plan was modified to use two-tailed tests for all analyses and to use analyses that adjusted for baseline differences in STI prevalences between the intervention and control cities.

The ELISA test for *C. trachomatis* and culture for *N. gonorrhoeae* proved very difficult to establish in some of the regional, government-run laboratories. A central laboratory was therefore established in Lima before the intervention began at the U.S. Naval Medical Research Detachment, and had to be relocated twice thereafter, first to the Peru National Institute of Health, later to a new laboratory at UPCH. The initial choice of centralized Roche AMPLICOR PCR testing, was followed by growing international evidence for non-specificity of this test for *N. gonorrhoeae*—a problem made more serious by the unexpectedly low prevalence of *N. gonorrhoeae*, further contributing to low positive predictive value of positive PCR tests.

Subsequent additional funding from USAID and NIH early in the intervention trial, and from the Wellcome Trust after the recommendations from the 2006 DSMB meeting, also further allowed us to substantially strengthen the intervention in the ten intervention cities by:

- Introduction of a health communication campaign to promote STD symptom recognition and appropriate early health-care seeking

- Social marketing in the intervention cities of a) dual purpose condoms for STD prophylaxis as well as contraception, and b) STD treatment packets marketed to pharmacies for treatment of urethral discharge in men and vaginal discharge in women

- During the last nine months of the intervention, the assessment in the intervention cities of the feasibility of mobile team outreach to male sex workers having commercial sex with men

- Also during the last nine months of the intervention, implementation of rapid point of care syphilis testing in both female and male sex workers

- Addition of HIV, HSV, HTLV, and hepatitis B serologic tests as secondary outcome measures, or for assessment of the prevalence of these sexually transmittable infections, or both

- Addition of Gen-Probe APTIMA NAAT testing of vaginal swab and male urine specimens from stored specimens from the 2002-03 baseline survey, and from the 2006 outcome surveys for *T. vaginalis*, allowing us to replace *T. vaginalis* culture with *T. vaginalis* NAAT tests as an outcome measure; and use of APTIMA assays allowed us to undertake confirmatory testing of specimens from participants classified as positive for *N. gonorrhoeae* or *C. trachomatis* by Roche AMPLICOR PCR testing.

II. Summary of protocol revisions

REVISED AIMS[A]

A. To evaluate the impact of a multi-component intervention on the composite prevalence of curable STIs *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, or syphilis seroreactivity (RPR ≥1:8, TP•PA confirmed) among female sex workers (FSW), and among men and women in the 18-29 year old general population,[B] in urban Peru. The intervention provided in the ten intervention cities consists of four modalities.

   (1) Pharmacy- and clinician-based strengthened STD syndromic management (through creation of the PREVEN Network), supported by social marketing of STI syndrome treatment packets, for urethritis in men, and for vaginal

[A] Antimicrobial susceptibility testing of *N. gonorrhoeae* was dropped after very low prevalence of *N. gonorrhoeae* was detected.
[B] Changed to general population instead of male military recruits and antenatal clients (UW IRB approval

discharge;[C] and a health communication campaign targeting recognition of STI symptoms and early health care-seeking[D]

(2) Mobile team outreach to FSW, including hard-to-reach FSW, to provide screening and treatment for STI[E]

(3) Periodic presumptive treatment of FSW by the mobile teams for vaginal trichomoniasis

(4) Promotion of condoms both for a) the general population, through social marketing of dual-purpose condoms (for STI prophylaxis and pregnancy prevention); and b) for FSW, through risk-reduction counseling with motivational interviewing emphasizing condom use with clients, supported by provision of up to 50 condoms every eight weeks

B. To develop and employ mathematical models of gonorrhea, chlamydial infection, syphilis, and trichomoniasis to explain the observed impact of the interventions, and facilitate transfer of the intervention to other locations.

STUDY DESIGN[F]

The study is an urban community randomized trial in Peru. Cross-sectional surveys were utilized to establish baseline and outcome prevalences in cities with mid-sized populations (between 50,000 and 300,000). Cities were matched with control cities based upon baseline prevalences of curable STIs [*N. gonorrhoeae, C. trachomatis, T. vaginalis*, and syphilis seropositivity (RPR ≥1:8, TP•PA confirmed)], and upon total population; and coastal, Andean or

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6/12/2002)

[C] First year of intervention only. USAID funded from October 2003 to October 2004

[D] Health communication campaign occurred from 2003 to August 2005

[E] A male sex worker intervention was added during the last nine months of the intervention (UW IRB approval 2/7/2006)

jungle region. Matched city pairs were randomly assigned to receive a multi-component intervention or no intervention using a random number generator program written in Splus (version 3.1). Follow-up surveys were conducted in years three and four after implementation of the intervention.[G]

STUDY SETTING AND POPULATION[H]

Cities in Peru are coastal, Andean, or jungle; are geographically dispersed; and because of limited transportation infrastructure, relatively isolated. The 30 cities other than Lima with populations ≥50,000 constitute the potential study units in our trial. Three of these were selected for a separate NIMH-funded trial, and therefore excluded from our trial. From the remaining 27 cities, we selected 24 for baseline studies from which 20 were selected, classified into matched pairs, and randomized to either serve as controls, or as intervention cities.

DESCRIPTION OF INTERVENTION

The Peru PREVEN Project: A Multicomponent HIV/Sexually Transmitted Disease (STD) Intervention

Intervention cities received a hybrid intervention targeting female sex workers (FSW) and the general population, consisting of four key modalities: 1) strengthening STD syndromic management for the general population, through training and support of pharmacy workers and referral networks of STD clinicians for improved STD recognition and management including STD/HIV prevention counseling; 2) mobile team outreach to FSW for STI screening and specific treatment; 3) presumptive periodic metronidazole treatment for trichomoniasis;, and 4) condom supply and promotion of condom use by FSW, plus social marketing of condoms for

[F] Re-designed to include two instead of three arms (UW IRB approval 6/12/2002)
[G] Initial Wellcome trust funding was adequate for a baseline survey and two years of intervention. An outcome survey in 2005 was funded by an NIH CIPRA grant. After DSMB review and recommendations in April 2006, the Wellcome Trust approved and funded continuation of the trial for another year, and final three year outcome surveys conducted in 2006.

the general population. The intervention timeline began in July 2003, scheduled to run for 30 months, ending in December 2005.

Intervention Modality 1: Strengthened STD Syndromic Management in Pharmacies, Physician Referral Networks, and MOH Referral Health Centers

Primary target audiences for this intervention included pharmacy workers, and physicians and midwives in private practice and health center settings. A census of pharmacies and boticas (drugstores not owned or managed by pharmacists, but allowed to sell all pharmaceuticals) and physicians and midwives was conducted in each of the intervention cities. In these cities, interventions targeted all of the pharmacies and private practice physicians and midwives who agreed to participate and clinicians from selected health centers. Pretested strategies included:

Prevention seminars for pharmacy workers: Four 90-minute training sessions with small groups who meet with two training specialists (a pharmacist and a midwife) emphasized recognition of STD syndromes, syndromic management guidelines, and referral of clients with genital ulcers or pelvic inflammatory disease to the network of trained clinicians. The final seminar/workshop provided training in motivational interviewing of clients by pharmacy workers to promote treatment of sex partners and condom use for casual and commercial sex. The curriculum, training materials, and reference materials were adapted from our pilot program in Lima.

"Prevention salespersons:" Pharmacists and midwives who received 50 hours of specialized training on STDs, health education, and counseling and communication skills conducted the prevention seminars and then made monthly visits to each pharmacy to answer questions and distribute educational materials for clients, including an STD prevention newsletter. These "prevention salespersons" represented an innovative approach to peer-mediated diffusion of

[H] No protocol change

information. In each of the cities we had an office with internet capabilities. The pharmacy working teams in each city were composed of a coordinator (the main prevention salesperson), and two to three assistants depending on the size of the city.

Continuing education for physicians and midwives in private practice: The training curriculum, which takes into account the adult learning process, self-taught learning, and distance education, consisted of: 1) an initial four hour induction workshop; followed by 2) one month of individual learning guided by a self-instruction manual and access to a training support system through the PREVEN webpage, telephone calls and visits from the local team; and 3) a final consolidation workshop. Based on a pilot project with 1,100 physicians in Lima, this training emphasized guidelines for STD syndromic management, patient counseling on condom use, and partner treatment. Trained physicians were evaluated and certified upon successful completion of the course and invited to participate in the PREVEN Network, a referral network of physicians, midwives, and pharmacies in each of the intervention cities. A directory listing trained and certified physicians and midwives was distributed to participating pharmacies. Follow-up with the physicians and midwives included monthly visits with the prevention salespersons, and a support system was provided through the PREVEN Network website and via telephone. Members of the PREVEN Network also received CME credit. A follow-up web-based course on STD syndromic management was created for physicians and midwives in clinical practice[I] in intervention cities. Leaders in each of the cities participated in discussion panels involving physicians, midwives, pharmacists and pharmacy workers, regional governments, PREVEN network leaders and other civil society members to seek local sustainability of the strategy started by the PREVEN project to control STDs and HIV, and other strategies towards the control of the HIV epidemic.

[I] Expanded to include online training course (UW IRB approval 4/06/05). (See Canchihuaman FA, et al, PLoS One. 2011 May 9;6(5):e19318.)

Counseling to pharmacy clients with STD: For men with a syndrome suggesting urethritis, women with symptoms of abnormal discharge, and for men and women with symptoms of genital ulcer disease seen in pharmacy settings, we adapted a promising client-centered intervention, pilot-tested a motivational interviewing training program with pharmacy workers in Lima, and developed a training video for STD/HIV prevention. The client-centered counseling primarily assesses the client's actual and perceived risk, reduces barriers to risk reduction, and supports behavior change and promotes partner treatment. These objectives reflect the 4 C's of syndromic management for STD: enhancing compliance; counseling for risk reduction; promoting condoms for high risk sex; and sexual contact referral and treatment. Treatment of vaginal discharge with metronidazole 2 g single dose aimed to cure trichomoniasis, and counseling was reinforced at the pharmacy level. Training pharmacy workers to recognize possible cases of PID and genital ulcer disease was also linked to training the workers to refer such cases for management to the members of the PREVEN Network.

STD symptoms recognition and early health care-seeking campaign[J]: This health communication campaign, carried out by the PREVEN team in each of the intervention cities, employed pamphlets, posters, local media, radios and local TV stations, with leaders from the PREVEN network and local authorities involved in the process. The main objectives of the campaign included recognition of urethral discharge as an STD and vaginal discharge as possibly an STD and knowledge of the resources for information (web page, trained pharmacies) and treatment (members of the PREVEN network).

[J] The Health Communication Campaign, on early STD symptom recognition and early healthcare-seeking from appropriate providers, was initiated in 2003 and continued, after a short pause, through August 2005. See Section V. Timeline.

Intervention Modality 2: Screening and Specific Treatment for STI in Sex Workers, and Periodic Presumptive Metronidazole Therapy for Sex Workers

Female Sex Workers: This intervention targeted all FSW, especially the most vulnerable, hard-to-reach subgroups of female sex workers (FSWs), typically young, socially-isolated, street-based FSW, and/or those who do not access available health services. Prior research in this group demonstrated high rates of STIs and frequent unprotected sexual intercourse.

A Mobile Outreach Team (MT) delivered this FSW intervention in each city. The MT consisted of a health professional and an FSW peer educator. The MT visited each identified sex venue within the city to conduct STI screening, and specifically treat any STI diagnosed through the screening. Using endocervical swabs in the 2002-03 baseline survey and self-obtained vaginal swabs[K] subsequently, STI screening by the MT included cultures for *Trichomonas vaginalis* processed in a government laboratory in the same city, and NAAT testing for *N. gonorrhoeae* and *C. trachomatis* infection, on specimens transported for rapid processing in a central laboratory in Lima, with prompt reporting of results by email. After seven days, the MT returned to the same sex work venue to deliver test results, providing specific treatment for those with positive tests for GC or CT, and for *T. vaginalis* if not previously treated presumptively (see Intervention Modality 3, below), and promoting consistent condom use and use of clinical services. Each intervention cycle was eight weeks long, during which the MT visits every identified sex work venue in the city. FSW intervention strategies included:

Promotion of 100% condom use in commercial sex: Mobile Team members educated and counseled FSW to consistently and correctly use condoms in all of their commercial sex encounters. The MT engaged "gatekeepers" (brothel and bar owners, managers, and pimps) to support the 100% condom use policy.

Promotion of screening and specific treatment of STI in FSW[L]: At baseline, an estimated 60-80% of FSW reported not utilizing existing clinical and counseling services provided by the Peruvian Ministry of Health's STD & HIV Control Program. As part of the FSW intervention, the Mobile Team:

1. Conducted bimonthly worksite visits for STI screening and treatment.[M] Mobile teams, consisting of a nurse-midwife and peer-outreach worker, collected self-obtained vaginal swabs. Swabs were mailed to Lima for central NAAT for *C. trachomatis* and *N. gonorrhoeae* with electronic return of results, and were carried to the local government laboratory for InPouch culture for *T. vaginalis*. Treatment and counseling was conducted on-site, allowing pathogen-specific treatment of FSW within one week of specimen collection. The mobile team revisited each work site within one week to provide test results, counseling, and treatment.

2. Promoted use of existing public STD services (CERETS and UAMP's) for diagnosis and treatment of any new symptoms of reproductive tract infection; for counseling and free condoms; and for screening for HIV, syphilis, and cervical and vaginal infections.

[K] Clinician obtained in year 1; self-obtained in follow-up years

[L] Male Sex Workers: During the last nine months of the study, a separate project was undertaken to assess the feasibility of mobile team outreach to male sex workers in the ten intervention cities. Mobile teams, consisting of a peer male sex worker, a health care worker, and a laboratory technician, were created to visit venues used by male sex workers and their clients. The intervention included not only risk reduction counseling, similar to that offered to FSW, distribution of 50 condoms per encounter, serologic testing for syphilis and HIV, and testing of first void urines for *C. trachomatis* and *N. gonorrhoeae*, with referrals for treatment as for FSW; but also baseline treatment of all with azithromycin 1.0 grams and with ciprofloxacin 500 mg. As this was a short-term feasibility study offered only during the last nine months of 2006, separate analysis of outcomes was planned, and not included with the FSW outcome analyses.

[M] Onsite rapid syphilis testing by a laboratory technician and referral for treatment initiated one year prior to the end of the project

Intervention Modality 3: Periodic Presumptive Metronidazole Therapy for FSW for Trichomoniasis

The MT offered periodic presumptive therapy with 2 g of metronidazole orally every two months, to each FSW able to forego consumption of alcohol for 72 hours.

Intervention Modality 4: Social Marketing of Condoms for the General Population, and Promotion of Condom Use, with Supplies of Condoms for Commercial Sex

In collaboration with a Peruvian non-governmental organization, APROPO, and funded by USAID, PREVEN used social marketing techniques (commercial marketing techniques adapted to meet social goals) to overcome barriers to condom use, with a marketing plan for sales, distribution, logistics management and promotion of condom use within the intervention cities.

APROPO placed a high quality/low cost condom, linked to the concept of double protection (protection against STDs and unwanted pregnancies) into the market in the intervention cities, through pharmacies – the first time in Peru that the concept of double protection has been introduced.[N] To avoid contamination of the control cities with this campaign, we designed the campaign to target only the intervention cities, limiting the distribution of this condom to these cities through negotiations with the local distributors, avoiding the use of nationwide mass media networks for promotion of the product and condom use, and using only local media (newspapers, radio stations) and a local network of volunteers to distribute pamphlets, posters and other promotional materials at discos, universities, internet cafes, pharmacies and other places where young adults gather.

[N] The Social Marketing Campaign was initiated in October 2003, by APROPO, and continued through October 2004. Afterwards, APROPO also marketed condoms in control cities.

Prevention activities in control cities

The control cities received no activities through this intervention related to training of pharmacists, pharmacy workers, physicians, nor midwives, in STD/HIV management, nor providing of STD treatment packets or health communicators campaign; no promotion or supply of condoms to sex workers; no MT outreach for screening and specific treatment for STI in FSW or male sex workers; and no presumptive treatment of FSW for trichomoniasis. The Ministry of Health's CERETS are special health centers offering services to FSW in both intervention and control cities. These services included: provision of free condoms, physical examination, screening, treatment and counseling for STD/HIV. Contingent on availability of supplies, the screening included wet mount of vaginal secretions for diagnosis of trichomoniasis, bacterial vaginosis, and candida; endocervical smears specimens collected for gram stains for gram negative diplococci (GC) and for GC culture; and syphilis and HIV serologic screening.. While focusing on FSW, these clinics also offer syndromic management of STDs to anyone from the general population seeking care.

OUTCOMES[O]

Primary Endpoint: Consistent with the primary study objective:

- Composite endpoint of infection with *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) and/or *Trichomonas vaginalis* (TV)[P] and/or syphilis (RPR ≥1:8 and TP•PA reactive as a surrogate for early syphilis) in the general population of young adults.

[O] Taken from the PREVEN TRIAL SUMMARY, Data Analysis plan, presented to the DSMB in 2006.

[P] With the DSMB report of unusually high TV prevalences based upon InPouch culture results as reported by some local laboratories, the DSMB suggested we remove *T. vaginalis* as an outcome measure. However, we were able to subsequently undertake re-testing of all baseline survey and 2006 survey specimens for *T. vaginalis* by NAAT using Gen-Probe's TV analyte-specific reagents with APTIMA General Purpose Reagents and therefore were able to retain *T. vaginalis* as the fourth component of the composite curable STI endpoint.

Secondary Endpoints: Consistent with the secondary study objectives:

- Combined prevalence of CT and/or GC and/or TV and/or syphilis (RPR $\geq$ 1:8) in FSW.
- Positive test for bacterial vaginosis and/or HIV[Q] in the general population and in the FSW.
- Reported condom use in the general population and FSW

PROCESS AND OUTPUT INDICATORS

Input measures included all resources and activities provided per city. Process and output measures monitored achievements within each arm of the intervention and included the following:

1. Percentages of pharmacies, pharmacy workers, and physicians/midwives completing training and continuing in follow up.
2. Monthly reports of STD cases seen at pharmacies and by private physicians and midwives in the intervention cities.
3. Evaluation by standardized simulated patients to follow changes in patterns of symptom recognition, counseling, and referral by pharmacy workers in intervention and control cities at baseline, 3, 6, and 18 months post training.
4. Sentinel surveillance of STD cases seen at pharmacies from control and intervention cities at 3, 9, and 18 months post-initiation of the intervention.
5. Number of FSW screened by the mobile team in each cycle.
6. FSW prevalence of CT by cycle and number of previous screenings by the MT.
7. FSW prevalence of GC by cycle and number of previous screenings by the MT.
8. FSW prevalence of TV by cycle and number of previous screenings by the MT.

[Q] Revision to add additional potential secondary outcomes: HSV2, HTLV, or Hepatitis B virus seropositivity; and HPV and *M. genitalium* (UW IRB Approval 1/31/2005)

9. Percent of FSW with positive CT, GC or TV adequately treated by the MT.
10. Percent of FSW reporting condom use with last client.
11. Quantity of selected drugs purchased by and dispensed in pharmacies, number of condoms distributed and sold.

SURVEY POPULATION

Cross-sectional surveys in two populations were implemented in order to determine the impact of the community intervention in the general population of young adults and in FSW. Secondary groups included FSW clients recruited at commercial sex venues (200 from each city) in 2002-03.[R]

General Population Survey (GPS)

The baseline 2002 GPS included men and women ages 18 to 29 years, residents of the 24 cities selected as candidates for the PREVEN study trial. The number of participants in baseline surveys for each of the 24 cities included 300 men and 300 women (250 random sample plus the first 50 partners identified as living in the households of these participants). In outcome surveys in 2005 and 2006 in the 20 cities participating in the intervention trial, the numbers of participants for each of the 20 cities included 300 men and 300 women, totaling 12,000. Recruitment timeframe totaled three months.

Inclusion Criteria

- Men and women ages 18 to 29 years at enrollment.
- Residence for a minimum of 6 months in the city where interview takes place.
- Lived in the city for at least 3 of the last 6 months.
- Spends at least 4 nights a week in the household where identified.

[R] Client surveys were discontinued after the baseline 2002-03 survey after found to not differ substantially from men in the general population.

- Mentally capable of providing informed consent and of responding to a questionnaire.
- Willing to participate in the survey by providing independent verbal informed consent.

Exclusion Criteria

- Inability to provide informed consent.

Participant Retention and Withdrawal

- No follow-up of participants required, and therefore no measures for retention were planned. Participants able to voluntarily withdraw from the study for any reason at any time.

FSW Survey

For this study, FSW were defined as women who offer their personal sex services at venues of sex work identified in the participating cities. The goal for each survey included 200 FSW enrolled in each of the cities (24 cities at baseline and 20 cities in outcome surveys), for a total of 4,800 at baseline and 4,000 in 2005 and in 2006. Enrollment was carried out in the same cities selected for the GPS conducted during 2002-03 for the baseline survey and 2005 and 2006 surveys, and according to the same timeline and timeframe as the GPS in the 2005 and 2006 surveys (3 months).

Inclusion Criteria

- Women found offering sex services at selected venues during pre-scheduled visits.
- Age 18 years or older.[S]
- Mentally capable of providing informed consent and responding to a questionnaire.
- Willing to participate in the survey by providing independent verbal informed consent.

[S] Revision to include sex workers ages 14 years and older (UW IRB approval 12/17/2004)

Participation in previous surveys, including our own previous STD surveys, does not preclude participation.

Exclusion Criteria

- Inability to provide informed consent because of obvious mental impairment due to the use of alcohol or drugs, or to other mental illness.

Participant Retention and Withdrawal

- No follow-up of participants is required, and therefore no measures for retention are planned. Participants may voluntarily withdraw from the study for any reason at any time.

SAMPLING AND SURVEY METHODS

General Population Survey (GPS)

The sampling frame for the study was based on data from the 1999-2000 pre-census household database compiled by the Peruvian National Institute of Statistics and Informatics (INEI). For our survey, INEI defines sampling sets with an average size of 80 households. These sets were divided into two equally sized clusters of approximately 40 households, so as to provide approximately 10 eligible participants per cluster. For each city, 108 clusters were selected using the random number generator of MS-Excel.

Field workers visited all households in the selected clusters, locating households with eligible members. A random sample of ten households with eligible members was selected from each cluster (or all households if less than ten). The interviewers returned to the selected households. If more than one household member met the inclusion criteria, the person with the most recent birthday was selected for participation. At this time, an appointment to meet the selected person was made. Selected participants then were interviewed to confirm eligibility

and to conduct the consent process to determine willingness to participate, and to complete the study procedures. Only in cases when eligibility of the participant could not be confirmed, the eligible person with the next most recent birthday was selected.

Field work was divided into nine periods lasting ten days each, corresponding to the nine independent samples selected for each city. Each interviewer covered four clusters during the first eight days of each period. Although the ninth day of the period was programmed for visits to participants not found during the initial visit, there was no set limit for the number of appointments made to find selected participants. Recruitment of participants continued until, at the end of a period, the gender-specific sample size has been reached or exceeded. We expected less than 100 clusters needed to reach the women's sample size, while in most cities, all 108 clusters must be visited to complete the sample size for men. Interviewers conducted field procedures wearing study gowns and carrying a backpack with survey materials and a small portable cooler containing frozen acrylamide gel bags.

A nine-day training course for field personnel included modules on: good clinical practice (GCP), ethics, basics of STDs, map handling, selection of participants, informed consent procedures, interviewing, sample collection and handling, and coding. The modules included formal lectures as well as demonstrations and hands-on laboratory practice. All course participants received a copy of an Interviewer's Manual for the study. For each city, a team of approximately three interviewers and a supervisor was selected. Trainees selected as supervisors also received a Supervisor's Manual. Health professionals, such as nurses, midwives, and medical doctors, comprised the interviewers and supervisors. Additionally, a team of six previously experienced regional coordinators and a national supervisor received training in this course. The course prepared all field personnel, supervisors, and coordinators, to be capable of conducting interviews.

FSW Survey

Local teams began field activities by performing a census of sex work venues (places where FSW meet their potential clients, e.g. brothels, bars or streets). The health promoter and key informants within each city identified these venues. Team members recorded the location and characteristics of the venues, as well as the approximate number of FSW expected to be found at the venue at different times during an average week. The census ended when all accessible sex work venues were recorded. Although it was expected that not all sex work venues would be identified by the census, extremely hard-to-reach venues were considered to be likely of relatively low epidemiological importance, as they tended to serve small closed circles of clients. At the end of the census, copies of the field work records were sent to the UPCH in Lima. In cities with more than 200 FSW identified by the census, a sample of about 200 women were selected by the time-location sampling method. In cities where fewer than 200 FSW were identified, the local team attempted to enroll all FSW identified.

Local teams visited commercial sex venues and invited all FSW present in that venue to participate in the study, and completed interviews and sample collection for this study in a single visit for all participants. After providing verbal informed consent, study participants completed an interviewer-administered questionnaire and underwent HIV and STD counseling and testing. All participants received a copy of the informed consent. Basic demographic data were collected from any FSW present at the venue during the visit but who do not meet the eligibility criteria or who refuse to participate.

Field work teams for each city included three counselors, a laboratory technician and a health promoter, usually a peer FSW. All counselors attended a centralized training workshop before the beginning of field activities. In addition to training in selection of study participants and the appropriate completion of the study questionnaire, the workshop included modules on

ethical issues including appropriate consent procedures granting confidentiality and avoiding any form of coercion. Laboratory technicians received training on appropriate techniques for sample collection, processing, and shipping, as well as on biosafety.

DATA COLLECTION

General Population Survey

Interview

Participants responded to a face-to-face questionnaire administered by the interviewer. The questionnaire addressed data on demographic and household characteristics, travel, migration, labor, health seeking behavior, marriage, contraception, knowledge about STDs and reproductive health. Afterwards, the participants responded to a self-administered questionnaire, exploring characteristics of the participant's sexual behavior.[T] A GPS Interviewers Manual of Procedures (MOP) provided detailed instructions to guide and standardize all study procedures across sites; it was changed in 2005 and 2006 to include the collection of data with PDAs.

Biological Sample Collection

After questionnaire completion, the interviewers explained procedures for sample collection, asking men to provide first void urine (about 15 ml) for STD tests in a 50-ml plastic container. In 2002, females received a rack with 1 polyester/plastic stick vaginal swab and two cotton/wooden stick vaginal swabs, to be self-applied in that order by the participant. The interviewer placed the polyester swab in an empty 2 ml cryovial. The first cotton swab was used to inoculate an InPouchTV® culture for *T. vaginalis* in the field; and the second cotton swab to prepare a vaginal smear on a glass slide in the

[T] The questionnaire for the 2002 survey involves a paper format. The general population survey questionnaire

field. Females unwilling to provide self-applied vaginal swabs were asked to provide urine samples.[U]

After collection of saliva[V] and urine or vaginal samples, the interviewers collected venous blood samples from men and women using a vacuum extraction system, using syringes when extraction of blood with the vacuum system was difficult.

Participants providing biological samples received a booklet with information about HIV and the HIV tests as pre-test counseling for HIV and a referral card to be used at a local health center to receive STD/HIV counseling and to receive test results.

Urine samples, dry polyester swabs in cryovials, and blood samples were placed in a cooler for transportation to the local laboratory facilities for specimen processing and transport. *T. vaginalis* cultures and glass smears were transported to the laboratory inside the backpack.

Consent forms, referral cards, and samples were labeled using preprinted self-adhesive labels with alphanumeric codes and barcodes. The same code was entered into the hand-held computer.

FSW Survey

Interview

FSW responded to a face-to-face demographic and sexual history questionnaire, after providing anonymous, verbal informed consent for study participation and for the storage of blood for future testing. The FSW Interviewers Manual of Procedures (MOP)

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changed from paper to PDA format prior to the second survey (UW IRB Approval 8/08/2005).

[U] During the 2006 survey, an extra polyester swab was collected prior to the other 3 swabs. This swab was placed by the interviewer in APTIMA® transport media and was used for the Gen-Probe APTIMA TMA for *N. gonorrhoeae*, *C. trachomatis*, and *T. vaginalis*.

provided detailed instructions to guide and standardize all study procedures across sites. When eligibility was not confirmed during the interview, the enrollment process was aborted promptly.

Biological Sample Collection

Blood and vaginal swabs were collected from consenting participants following the same procedures described for the GPS, though in 2002, a clinician obtained an endocervical swab and vaginal swabs via speculum exam.[W] Participants received a referral card to be used at the local health center to obtain their laboratory test results, and received treatment if necessary. They also received condoms.

LABORATORY METHODS

The urine sample, a dry polyester swab in a cryovial, and the blood sample were placed inside a cooler for transportation to the local laboratory. A cotton swab was used to inoculate *T. vaginalis* culture medium (InPouchTV, Biomed, Oregon, USA). A second cotton swab was used to prepare a glass slide for Gram stains. *T. vaginalis* culture specimens were transported at room temperature and placed in an incubator upon arrival to the local laboratory, where they were examined for motile *T. vaginalis* forms daily for 5 days. Glass slides were transported to the local laboratory to be stored for up to 7 days. Serum and urine aliquots, as well as well as prepared glass slides, were shipped weekly to the central laboratory in Lima. All specimens for NAAT were stored at −20℃ and shipped frozen to the central laboratory for testing. Upon arrival to the central laboratory, the dry polyester swab was eluted in 2 ml of 2SP and stored at -−20C°until processed.

[V] Saliva testing for HIV discontinued prior to second survey (UW IRB Approval 8/08/2005)
[W] For the 2005 and 2006 surveys of FSW, clinician swab collection discontinued and changed to self-obtained vaginal swabs only (UW IRB Approval 8/08/2005)

Gonococcal Infection

REVISION[X]: Specimens from 2002-2003 and 2005 surveys reported as gonorrhea positive in the AMPLICOR assay, according to manufacturer's instructions, were further tested by the Gen-Probe APTIMA assay, utilizing specimens suspended in 2SP, allowing final classification as positive or negative based upon the APTIMA assay. Specimens from the 2006 survey were tested first by the APTIMA assay and then all positive specimens were tested by the AMPLICOR assay. Positive results are confirmed by APTIMA testing of specimens suspended in 2SP. Gonococcal infection is defined by positive AMPLICOR plus positive APTIMA assays.

Chlamydial Infection

REVISION[Y]: Specimens from the 2002-2003 and 2005 surveys reported as indeterminate by Roche AMPLICOR were tested by the Gen-Probe APTIMA Combo 2 Assay (Gen-Probe, San Diego, CA). The 2006 survey employed initial testing by the Gen-Probe Aptima assay utilizing the specimen collected in APTIMA transport medium; all positive specimens then were tested by the AMPLICOR assay performed on the specimen eluted in 2SP.

Results from the 2002-03 and 2005 surveys are considered positive if initially AMPLICOR positive; or if initially indeterminate and subsequently positive in the APTIMA assay. Specimens from the 2006 survey positive by both assays are considered positive. Those specimens yielding discordant results undergo APTIMA re-testing of specimens suspended in 2SP, for final classification as positive or negative. For these specimens, chlamydial infection is

[X] Rationale for revision: Target sequences in the methyltransferase gene of *N. gonorrhoeae* used in the AMPLICOR assay have been shown to be present in other Neisseria species[4] and "false-priming" has been suggested with lactobacilli in this assay[5]. In interim analysis, prior to unblinding of the data, we found that while 70% and 48% of urine and clinician-collected samples were APTIMA positive, respectively, only 12% of self-collected vaginal swab AMPLICOR positive specimens were positive for gonorrhea in the APTIMA assay. Those specimens AMPLICOR positive and APTIMA negative were much more likely to have had lower AMPLICOR optical density readings than were specimens positive in both tests (data not shown). This finding is consistent with previous reports associating apparent false-positive AMPLICOR results with lower test signal strengths.

[Y] Although studies have shown quite similar sensitivity and specificity of the Roche AMPLICOR and Gen-Probe APTIMA assays for chlamydial infection, the AMPLICOR assay yields an important proportion of specimens

defined by positive results in the APTIMA assay performed on the 2SP-suspended specimen. (See Appendix A: Laboratory Flow Charts).

Trichomonas vaginalis

REVISION[Z]: The Gen-Probe TV-TMA assay was used to test all 2002-03 and 2006 vaginal swab samples and to validate positive and a subset of negative InPouch culture results from the 2005 surveys. The final results showed high positive predictive values of InPouch positive cultures, in terms of APTIMA TV-TMA confirmation, in 17 of 20 cities, but no correlation of InPouch with TV-TMA in three cities; and showed low sensitivity of the InPouch cultures (approximately 50%) in relation to TV-TMA results in all cities.

Syphilis seroreactive

The quantitative RPR test was utilized; specimens reported as RPR reactive were confirmed by the TP•PA test. As a surrogate for possible early syphilis, syphilis seroreactivity is defined by an RPR reactive ≥1:8 with a reactive TP•PA.

HIV

Serum samples were tested by HIV ELISA; dually ELISA positive specimens were confirmed by Western blot. Aliquots of specimens reported as Western blot indeterminate in Lima are shipped to the University of Washington Retrovirology Laboratory for confirmatory Western blot testing.

Bacterial Vaginosis

Gram stain smears were utilized for assessment of bacterial vaginosis. Bacterial vaginosis is defined by a Nugent score of ≥7.

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reported as "indeterminate" (equivocal or inhibited)[6,7]

[Z] Rationale for protocol change: The 2006 DSMB review described unusually high prevalences of trichomoniasis in some of the 20 study cities.

III. Statistical considerations

Descriptive methods (e.g. means, prevalences) and plots (e.g. STI prevalences over time) form the bases of initial analyses. We summarized participation rates, sample sizes, demographics (e.g., age, gender, marital status, education, etc.) and curable STI prevalences (*Chlamydia trachomatis*, *N. gonorrhoeae*, syphilis seropositivity, or vaginal trichomoniasis, HIV) overall, by city and by intervention arm for each survey (2002-03, 2005, 2006; FSW and GP). We also summarized condom use rates in the GP overall, by gender, by arm and among the following subgroups—men with commercial sex partners in the last year, men with male sex partners in the last year. For the baseline survey, we added testing of samples of sera for HSV-2 and HTLV seropositivity.

PRIMARY ANALYSIS[AA]

The unit of analysis will be the city (i.e., composite curable STI prevalence at the city level). This approach is appropriate since the sample size is approximately the same from city to city. The primary analysis of intervention efficacy will be based on the model

$$d_i^O = b_0 + b_1 d_i^B + \varepsilon_i \qquad \text{(M1)}$$

where d_i^O is the difference in the primary composite STI endpoint [gonorrhea, chlamydia, vaginal trichomonas, and syphilis seroreactivity (RPR ≥1:8, TP•PA confirmed)] between the intervention and control communities (control – intervention) of the *i*'th pair in the final (2006) outcome survey, d_i^B is the corresponding difference in the baseline (2002) survey and ε_i is a random error. The hypothesis of interest is

Ho: $b_0 = 0$ (no intervention effect) (H1)

[AA] Primary and secondary analysis descriptions taken from "PREVENanalysisPlan_23.01.07.doc" dated 1/24/07 and updated 3/18/09

Ha: $b_0 \neq 0$ (intervention effect)

Note that if d_i^B was not included in the model, this analysis would be equivalent to a paired t-test. The d_i^B term is included in the model to control for differences in STI prevalence at baseline (which may occur in spite of the matching).

Least squares regression will be used for model fitting. The standardized test statistic $\hat{b}_0 / se(\hat{b}_0)$ from this analysis has a t-distribution with 8 degrees of freedom. A p-value will be reported for the hypothesis (H1) and a type I error rate (α) of 0.05 (two-tailed) will be used to reject/fail to reject the null hypothesis (H1).

The endpoint in the primary analysis will be the composite curable STI endpoint [combined gonorrhea, chlamydia, vaginal trichomoniasis, or syphilis (RPR ≥1:8, TP•PA confirmed)] as measured in the GP.

SECONDARY ANALYSIS

The analysis outlined above was used for the following endpoints:

- Composite curable STI prevalence [gonorrhea, chlamydia, vaginal trichomoniasis, or syphilis (RPR ≥1:8, TP•PA confirmed)] in FSW
- Individual STI in GPS
- Individual STI in FSW

POWER CALCULATIONS[BB]

In Table 2 we present power calculations for testing for a 30-50% reduction in the primary STD outcomes between the treatment and control communities based on the random

[BB] Power calculations taken from "PREVEN trial summary.doc" dated 3/7/06 and presented for DSMB review in April 2006.

population survey of 600 men and women per city. The FSW population is considered separately. These calculations make use of the standard sample size/power formula for matched community randomized trials, as shown below.

$$n = 2 + \frac{(Z_{1-\alpha} + Z_{1-\beta})^2 \left[p_1(1-p_1)/f + p_2(1-p_2)/f + k^2(p_1^2 + p_2^2) \right]}{(p_1 - p_2)^2}$$

In this formula p_1 and p_2 are the disease prevalences in the treatment and control communities, respectively, *k* is the coefficient of variation of disease prevalence between communities, *f* is the number of individuals per community and *n* is the number of communities. The Z's in the formula represent standard normal quantiles for appropriate type I and type II error rates. Disease prevalence in the control communities and the coefficient of variation are based on the baseline survey data.

Although the investigators cannot be formally blinded to the treatment assignments, only pooled results (over the two study arms) will be presented by the statistician at investigator meetings.

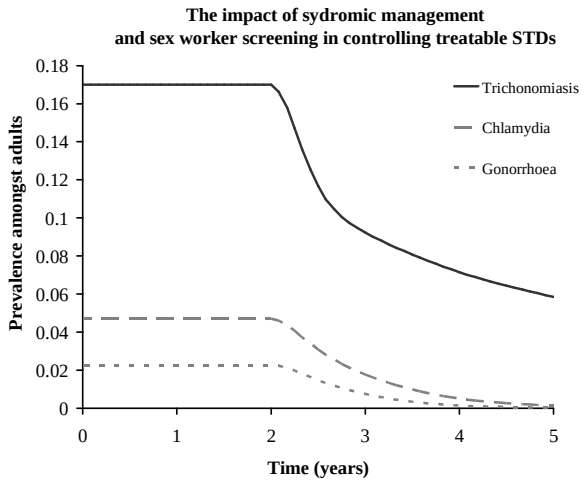
Table 2. Power for comparing treatment (*n* = 10) and control communities (*n* = 10) given number per community, prevalence, α = 0.05 (1-tailed), coefficient of variation = .10 and indicated reduction in disease in treatment cities. Number of GPS results is reduced from 600 to 500 to allow for refusal to provide biologic samples.

	Number per community			Percent reduction		
	General population	FSW	Prev	30 Percent	40 Percent	50 Percent
Any STD	500		.10	.99	.99	.99
		200	.14	.87	.98	.99
Chlamydia	500		.04	.86	.98	.99
Trichomonas	250		.05	.72	.92	.99
Syphilis	500		.01	.41	.63	.81
HIV	500		.005	.27	.40	.57

MATHEMATICAL MODELS

Mathematical models will be used to help distinguish the effect of different aspects of the interventions on the epidemiology of the different STDs. Data collected within the proposed studies and in other studies within Peru (e.g., 1996, 2000, and 2004 DHS surveys) on social, demographic, and behavioral variables (including number of sexual partnerships, frequency of unprotected sex, and patterns of sexual mixing) will be used by Imperial College investigators to develop quantitative models of STD transmission that will aid in understanding the epidemiology of STD in Peru. The observed risk behaviors of sex workers and their clients, and the proportion of young men using FSW, will be included in models to determine relative contributions of treatment versus behavior change. Meta-population models describing the transmission of STDs within and between cities will be developed to determine the importance of geographic reservoirs in maintaining the infections within the population. One hypothesis we

will explore is that more isolated communities will be less prone to reintroduction of infections, making interventions more successful.



We employed modeling to assess the potential impact of the proposed interventions on STD rates in the population. We assume the pharmacy-based intervention decreases the mean duration of symptomatic infections to 2 weeks for 70% of men and women while the CSW intervention decreases the mean duration of asymptomatic infections in sex workers to 2 months i.e., the sex workers are screened on average 2 months after becoming infected. These results (Figure) support the proposed 3-year intervention period.

IV. Human subjects considerations

ETHICAL REVIEW

Subsequent to initial review and approval, the responsible local IRBs or Ethical Committees (UPCH, UW, and NMRCD) reviewed the study annually. The investigators made safety and progress reports to the IRBs/ECs annually, and will report within three months of study termination or completion. These reports included the total number of participants enrolled in the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others.

INFORMED CONSENT

To protect anonymity, participants provided verbal rather written informed consent to complete a questionnaire and to provide biological specimens for STD/HIV testing. Consent also asked permission to keep specimens for future studies. The interviewers recorded in the consent process guide whether the participant agreed to provide questionnaire data and/or samples for the study, and whether they consented for HIV testing and/or storage of samples

for future studies. This information was transferred to electronic databases that are used to verify that each specimen collected has a valid informed consent form for the study, before the diagnostic tests are performed.

CONFIDENTIALITY

All data are anonymous. We did not collect names of individuals or ask for signatures. Documents and specimens were labeled with consecutive alphanumeric codes, with prefixes indicating population and city, and suffixes indicating the type of document or specimen Participants were given a card with their codes and instructions of where and when to go to seek their test results and additional counseling anonymously at a regional health center. As per the Peruvian law, participants received face-to-face counseling at the health centers before receiving test results.

RISKS AND BENEFITS

Study participants may experience the following:

- Discomfort during phlebotomy. Participants may experience minor pain, bleeding, and/or bruising at the venipuncture site, and/or vasovagal reactions. Some participants may experience lightheadedness.
- Embarrassment and concern when answering questions about risk factors of HIV and STD infection. Subjects may choose to not answer any question that makes them feel uncomfortable.
- Distress at finding out that they have HIV or another STD. All participants will receive pre-test and post-test counseling.
- Discrimination if others learn participants' HIV status. To help protect human subjects, all study staff will receive training in safeguarding confidentiality as well as in the

protection of human subjects. All information on participants will be kept extremely confidential and will not be shared with non-investigators.

- Other unforeseen potential harms: Although potential harm to participants in this study is expected to be minimal, the known risk will be thoroughly explained to participants as part of the informed consent process.

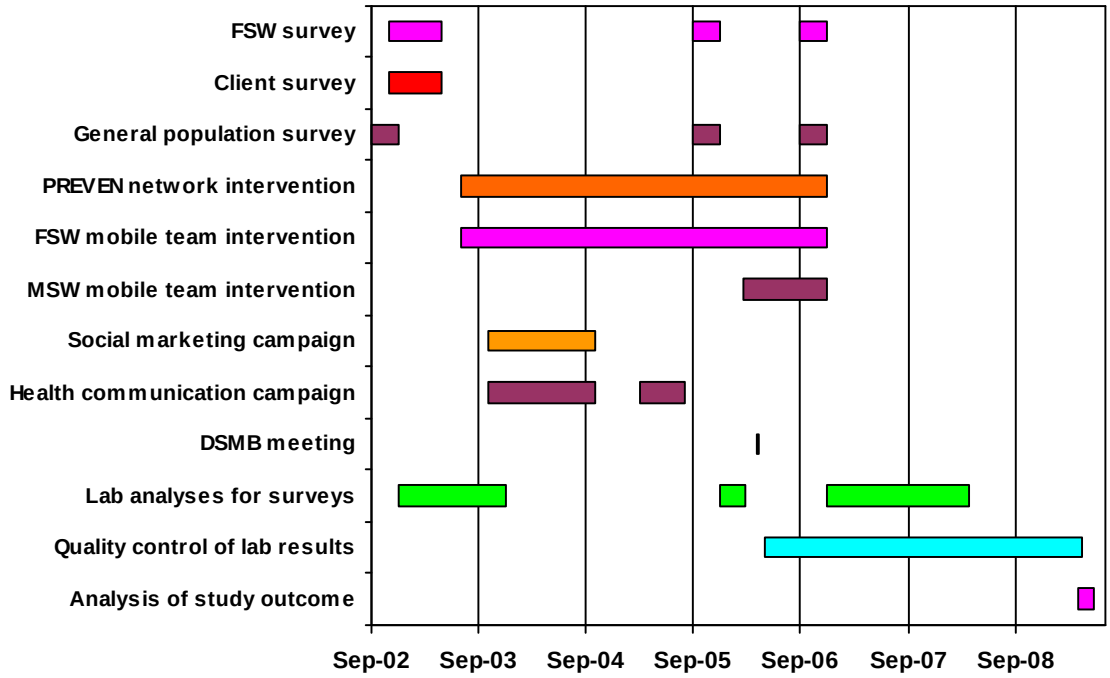
Although adverse events in this study are expected to be uncommon, the known risks are still thoroughly explained to participants as part of the informed consent process. All interviewers, counselors, and laboratory technicians record any unforeseen adverse event in their daily activity log and report it to their supervisors/coordinators. The supervisors/ coordinators generate electronic reports via the study's web page. The report is accessible to the core team which reports it to the local IRB/EC.

Benefits include receiving screening and treatment if indicated for STIs. Pharmacy staffs receive training to improve management of STIs and referrals. The FSW intervention improves coverage and services to a high-risk group who usually do not have access to high quality care. If the model proves effective, the intervention could be expanded to other cities. Participants for both surveys are not monetarily reimbursed, but instead receive small gifts (study caps and T-shirts for GPS participants, and 15 condoms for FSW survey participants) as compensation for their participation.

V. Timeline

We initially planned to maintain the interventions for 2.5 years, but with additional funding from the Wellcome Trust, and upon recommendation from the DSMB, we were able to continue the PREVEN Network and FSW mobile team interventions for approximately 40 months, including six months of scale-up of these interventions, and 36 months of the full

intervention.[CC] Interim surveys were completed in Year 3 and final outcome surveys were completed in Year 5.



[CC] Updated timeline with additional grant funding from Wellcome Trust Health Consequences of Population Change (UW IRB Approval 8/11/2006)

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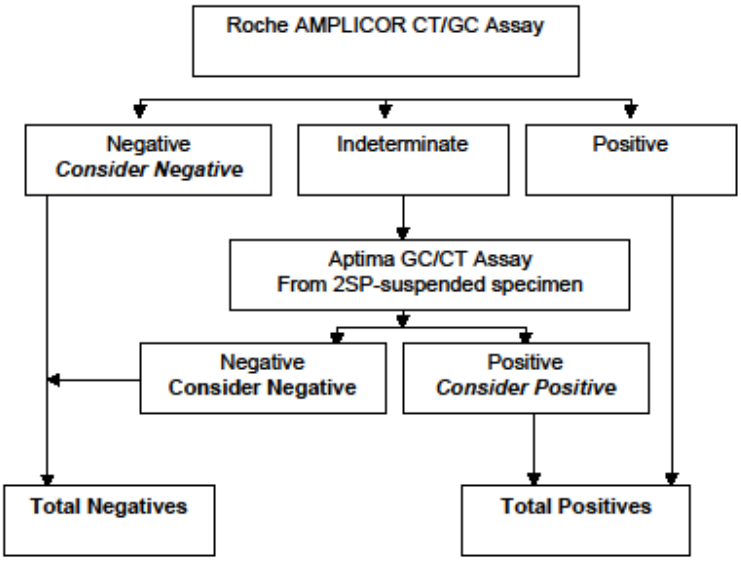
Appendix A: Laboratory Flow Charts

Chlamydia: 2002

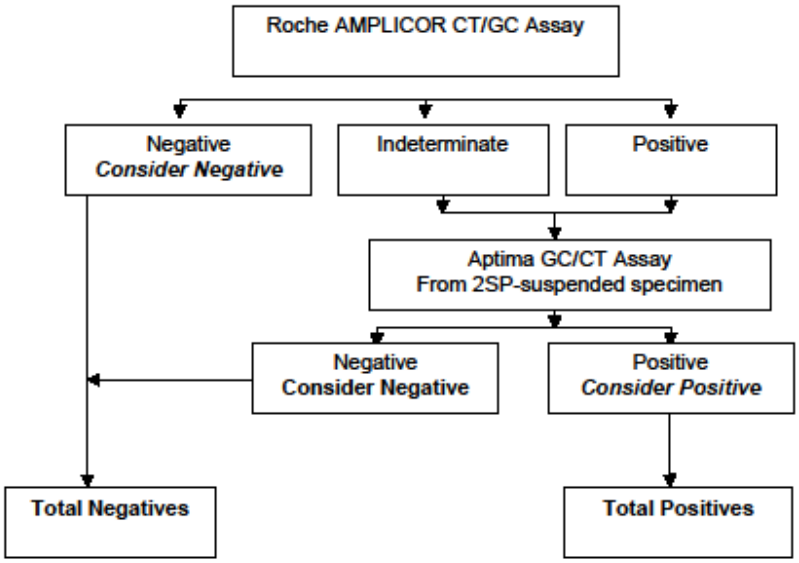


Definitions

Positive – Positive in the Roche assays or positive in the Aptima assay performed on the 2SP-suspended specimen from individuals indeterminate in the Roche assay.

Negative – Initially negative in the Roche assay or negative in the Aptima assay subsequently performed on the 2SP-suspended specimen.

Gonorrhea: 2002

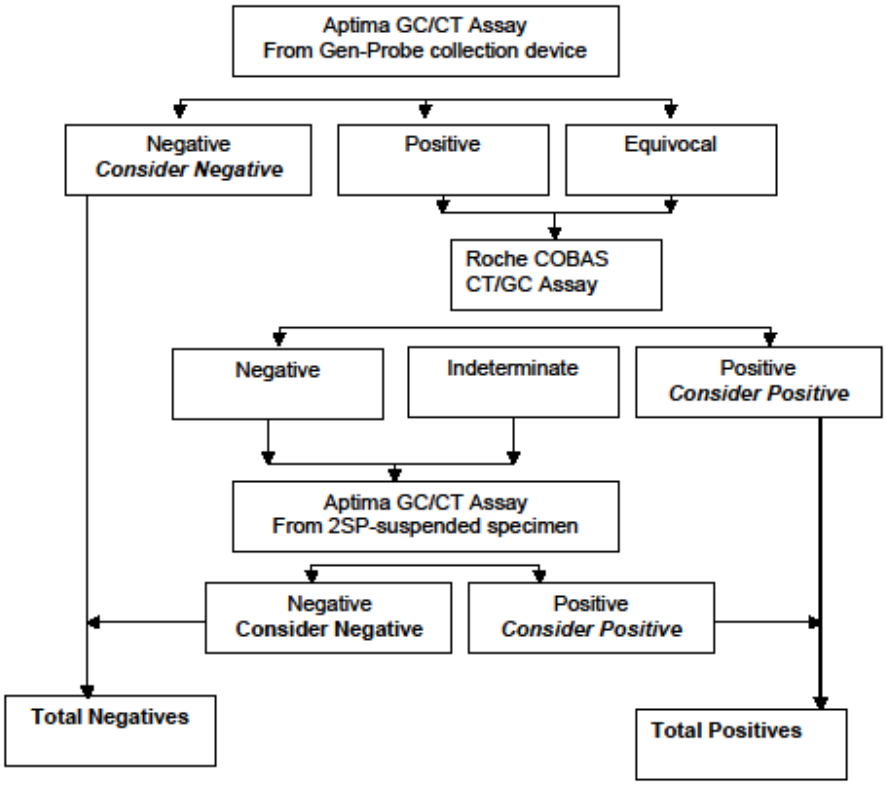


Definitions

Positive – Positive in both the Aptima and Roche assays or positive in the Aptima assay performed on the 2SP-suspended specimen from individuals indeterminate in the Roche assay.

Negative – Initially negative in the Roche assay or negative in the Aptima assay subsequently performed on the 2SP-suspended specimen.

Chlamydia and Gonorrhea: 2006

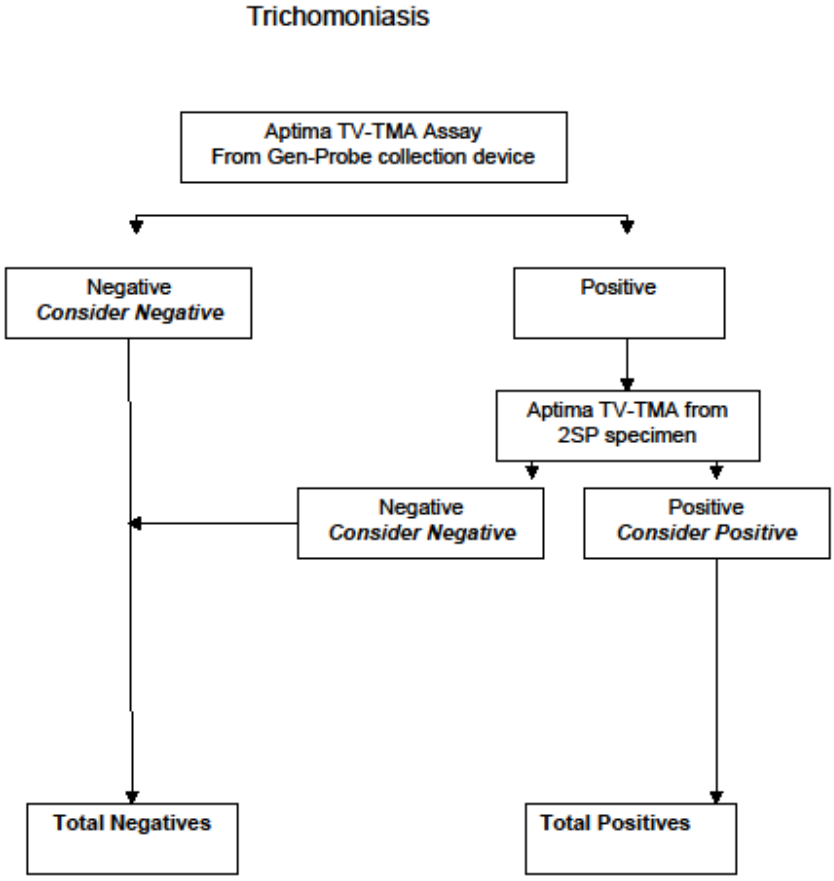


Definitions

Positive – Initially positive in both the Aptima and Roche assays or positive in the Aptima assay performed on the 2SP-suspended specimen from individuals positive or equivocal in the Aptima assay and negative or indeterminate in the Roche assay.

Negative – Initially negative in the Aptima assay or negative in the Aptima assay subsequently performed on the 2SP-suspended specimen.

Trichomoniasis 2006



Definitions

***Trichomonas vaginalis* positive** – Initially positive in the Aptima TV-TMA assays performed on the specimen collected in the Gen-Probe collection media and subsequently positive in the Aptima TV-TMA assay performed on the 2SP-suspended specimen.
***Trichomonas vaginalis* negative** – Initially negative in the Aptima TV-TMA assay or negative in the Aptima assay subsequently performed on the 2SP-suspended specimen.

PREVEN TRIAL SUMMARY PRESENTED TO DSMB, APRIL 2006

Collaborating Institutions:
Imperial Collage, London, England (Geoff Garnett, PI)
Universidad Peruana Cayetano Hereida, Lima, Peru (Patricia Garcia, PI)
University of Washington, Seattle, USA (King Holmes, PI)

Purpose: To evaluate the impact of a hybrid HIV/STD intervention on a high-risk group of female sex workers (FSW) and on the lower-risk young adult general population.

Design: Two cross-sectional surveys

Population: a) Men and women 18 to 29 years of age from the general population and
b) Female Sex Workers (FSW) aged 14 years and older

Study Sample: a) 6,000 men and 6,000 women for the General Population Survey (GPS) and
b) 4,000 FSW

Treatment Regimen: None

Study Duration: Three months

Primary Objective:

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalence of gonorrhea and/or chlamydia and/or trichomonas and/or syphilis (RPR $\geq$ 1:8) at the population level.

Secondary Objectives:

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalence of bacterial vaginosis and HIV infection.

Endpoints or Outcomes:

- **Primary Endpoint:** Consistent with the primary study objective: confirmed, positive test for chlamydia and/or gonorrhea and/or trichomonas and/or syphilis.

- **Secondary Endpoints:** Consistent with the secondary study objectives: confirmed, positive test for bacterial vaginosis and/or HIV.

Study Sites: Twenty midsized cities in Peru

Ayacucho	Chimbote	Huaraz	Iquitos	Tacna
Barranca	Chincha	Huanuco	Juliaca	Talara
Cajamarca	Cuzco	Ica	Piura	Tarapoto
Cerro de Pasco	Huancayo	Ilo	Pucallpa	Tumbes

Funding sources:

Activities	Funding source
Baseline surveys of: 1) the young adult general population, 2) FSW & 3) clients of FSW	Wellcome Trust-Burroughs Wellcome Fund Infectious Disease Initiative
Intervention Activities: STD syndromic management and provider trainings	WT-BWF
FSW Mobile Team treatment and outreach	WT-BWF
Social marketing of condoms	USAID
Outcome surveys of: 1) the young adult general population & 2) FSW	NIH CIPRA Project grant

The PREVEN Trial Design Overview

The primary aim of this trial is to evaluate the impact of a hybrid STD/HIV preventive intervention CRT on the prevalence of sexually transmitted infections (STIs), gonorrhea, chlamydia, trichomonas, and syphilis, in a high-risk group of female sex workers (FSW) and in the lower risk young adult general population. The study is a community randomized trial of 20 medium sized cities in Peru. The primary outcome (STI prevalence) will be measured in two cross sectional surveys, one in a population of FSW and the other in the general population of young adults, in each of the 20 Peruvian cities. The surveys will also provide us with estimates of the fraction of HIV cases that are diagnosed and treated in existing programs (Ministry of Health, Army, Navy, Police health services, Social Security system and other programs in Peru), the prevalence of HIV viremia, the prevalence of bacterial vaginosis (BV) and risk behaviors. In secondary analyses we will use this information to identify characteristics of bridging populations and determining the structure of sexual networks. This information will be directly useful for planning nationwide programs that integrate prevention and treatment.

The remainder of this document contains information on the baseline surveys we conducted in 24 cities in 2002 (subsequently reduced to 20 cities for the trial) (section 1), a description of the randomization and intervention (section 2), a description of the study populations (section 3), and further information on survey study procedures (section 4), risks to participants (section 5) and statistical methods (section 6). Figure 1 presents the timeline for the study activities including the 2002 baseline surveys, implementation of the hybrid intervention, and the 2005 surveys of FSW and GPS of adults 18-29 years of age.

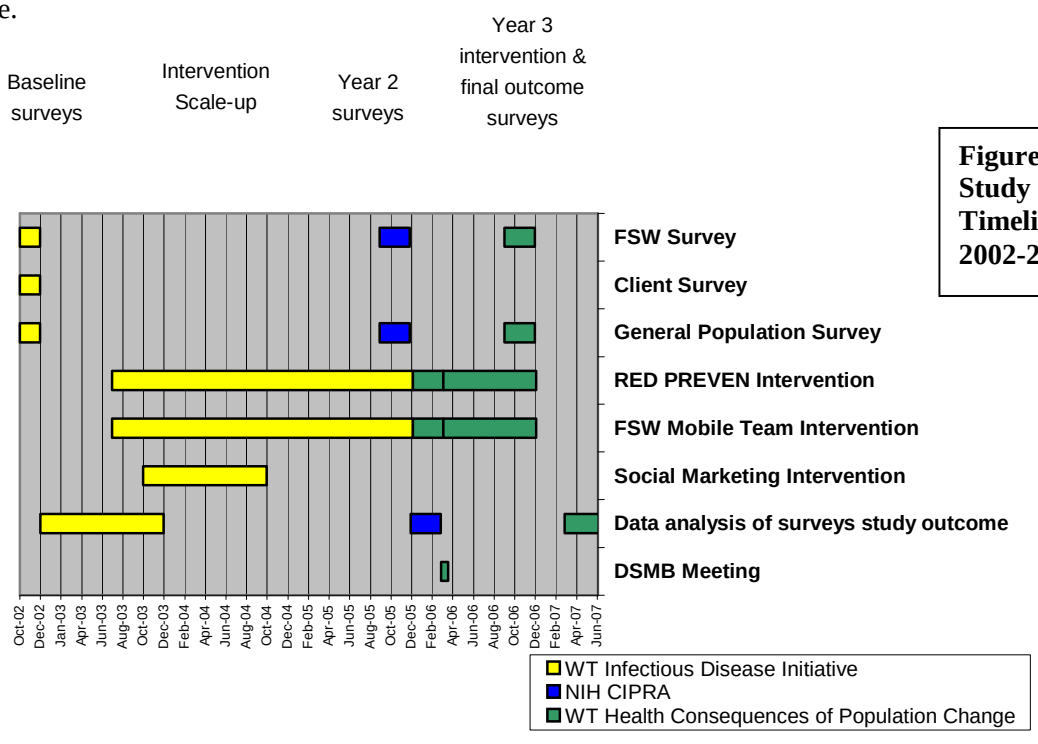


Figure 1. Study Timeline 2002-2007

1. 2002 & 2003 BASELINE SURVEYS IN PERU

Cities in Peru are geographically diverse (coastal, Andean, or jungle), and relatively isolated due to limited transportation infrastructure. The 30 cities other than Lima (which was excluded from the trial because of its disproportionate size) with populations ≥ 50,000 constituted the potential study units in our trial. Three of these have been selected for a separate National Institute of Mental Health (NIMH)-funded trial and were thus excluded from our study. From the remaining 27, we identified 24 for baseline studies (figure 2).

Figure 2. Map of Peru with 2002 Baseline Cities Identified

Support from the Wellcome Trust was used to conduct baseline pre-intervention surveys of three populations. These surveys included a national household-based general population survey (GPS) of 18-29 year-old adults and national surveys of FSW and clients in the 24 cities considered for inclusion in the hybrid intervention trial. These surveys gathered baseline behavioral and biological information to demonstrate their feasibility and aid in the study design.

1.1 General Population-Based Survey in 2002: Sampling, Participation and Responses of Young Adults.

The baseline random household sample included 17,055 18-29 year-old adults (50.3% of whom were females) from 24 cities during Oct.-Dec. 2002. Dr. Carcamo (a co-investigator for this project) and the INEI (Peruvian National Institute for Statistics & Informatics) used year 2000 census data

to randomly select households for the GPS by cluster sampling (each cluster was an average of 40 households). A random sample of clusters was selected for each city. Then a census was conducted for each household in the selected clusters and a random sample of households with eligible members was selected. Within each selected household, one eligible individual (male or female, aged 18 - 29, living in the city for at least 6 months) was randomly selected (based on birth date). Finally, for the 15,492 participants who completed the questionnaires, a consecutive sample of 2,307 same-residence sex partners was also enrolled. The sampling method and study procedures are described in greater detail in Sections 3.1 and 4.1 respectively.

The selection and training of interviewers and the development and pilot testing of questionnaires received careful attention during the preparation phase of the survey. For the GPS, interviewers visibly displayed logos of the INEI and the UPCH (Universidad Peruana Cayetano Heredia), both of which are well recognized and respected in the country. They visited households up to 5 times until selected individuals were found at home and scheduled revisits at times convenient for interviewees. To ensure confidentiality and privacy they used the self-administered questionnaire "voting box" or "secret ballot approach." Participants were offered tokens of appreciation for their participation (e.g., a T-shirt or cap). Anonymity of participants and their results was secured through the use of anonymous, verbal consents; participants' test results were provided via coded identifiers.

Participation rates compare favorably with participation rates from the U.S. National Health and Social Life Survey, where 2 percent of participants were not contacted and 20% did not complete the questionnaire. Of the 15,492 participants who completed a questionnaire in the GPS, 99.7% answered the self-administered risk behavior section. Non-response rates were low even for sensitive questions (see Table. 1). As part of the analysis of the baseline survey data, we have been able to identify problematic questions, and improve the survey instrument for the 2005 GPS.

Of those completing our questionnaires, 86% provided biological samples. Biological specimen management was done using specimen bar coding and a management tracking system developed with the US-NMRCD laboratory, which handles all specimens for this trial. With this system, only five specimens were lost in transport. We collected the socioeconomic status (SES) level for the GPS clusters, ages and genders. These variables are currently being reviewed to identify systematic differences between participants and people refusing to participate. Comparison of information for non-participants and participants may provide insight into their differences, and permit weighting for participation bias in analyses. Such weighted estimates can provide insight into the extent of possible bias and may allow calculation of more realistic prevalence estimates of particular behaviors.

Table 1. Non-response/No. returning questionnaire

	Male (7505)	Female (7801)
Ever had sex	352/7485 (4.7%)	388/7781 (5.0%)
Age at sexual debut (if experienced)	335/6275 (5.3%)	355/5885 (6.0%)
Anal sex with last partner (if experienced)	622/6276 (9.9%)	498/5885 (8.5%)

STD prevalences for young adults in the general population: Among men ages 18-29, 4.0% had chlamydia and 0.3% had gonorrhea; 0.4% tested positive for HIV, which was significantly associated with a high number of lifetime partners ($p < 0.001$), recognizing at least one of the last three partners as casual ($p < 0.001$), reporting sex with another man in the last year ($p < 0.001$)and reporting unprotected sex with a FSW in the last year ($p < 0.01$). Among women ages 18-29, 6.8% had chlamydia, 0.8% had gonorrhea, 5.2% had trichomonas and 0.1% tested positive for HIV.

1.2 FSW & Client Surveys in 2003: Sampling, Participation and Responses

Prostitution is legal in Peru and is regulated by the national government and local authorities. Traditionally, the MINSA (Ministry of Health) anti-venereal disease clinics have offered periodic checkups to FSWs. Starting in 1996, the MINSA established a nationwide system for improved voluntary periodic clinical examination and treatment directed to FSWs, setting up STD reference centers in all the main cities of Peru. The system also included the use of peer educators visiting sex work venues to promote safer behaviors and the use the clinical services offered by the government. In 1998 the MINSA started a sentinel surveillance system directed to high risk populations, including FSWs. Nationwide surveys of FSWs have been carried out in years 1998, 2000, and 2001. These surveys included mostly FSWs using the services of the STD reference centers. A 2003 survey was conducted in collaboration with the PREVEN study, and was carried out at sex work venues. The sampling method and study procedures are described in greater detail in Sections 3.2 and 4.2, respectively.

During January-March 2003, Dr. Campos (a co-investigator for this project), the OGE (Peruvian General Office of Epidemiology), and the regional AIDS and STD Offices undertook surveys of FSW and their clients in the same 24 cities. Our 2003 time-venue baseline survey of FSW and clients achieved 87.1% (range 63% - 100% per city) participation from FSW and 82.8% participation from clients. Of 4494 FSW participants, nearly all responded to sensitive questions, 98.8% provided blood samples, and 97.4% provided vaginal swabs. Of 4504 client participants, 97.7% provided blood and 95% (those able to void) provided urine.

STD prevalences for FSW and Clients of FSW: 14.5% of FSWs had chlamydia, 3.5% had gonorrhea, 7.9% had trichomonas and 3.9% were RPR reactive. Data suggest that approximately 0.25% of participating FSWs had acquired HIV infection within the last year.

Among clients of FSW, 4.0 % had chlamydia and had 0.2% gonorrhea. RPR reactivity was found in 5.6% of those with and 2.2% of those without recent history of genital ulcer ($p<.05$). Data suggest that approximately 0.25% of the clients acquired HIV infection within the last year.

2. RANDOMIZATION AND IMPLEMENTATION OF THE HYBRID HIV/STD INTERVENTION

From the 24 cities that took part in the 2002 general population and 2003 FSW and client surveys, twenty were selected to participate in the urban CRT and randomized into the following intervention and control groups (see in Table 2).

Table 2. Matched city pairs for the PREVEN project

	Matched pairs	
	Control City	Intervention City
1	Chimbote	Piura
2	Ilo	Tumbes
3	Ayacucho	Cajamarca
4	Tacna	Ica
5	Huaraz	Cerro de Pasco
6	Huancayo	Cuzco
7	Talara	Chincha
8	Tarapoto	Huanuco
9	Iquitos	Pucallpa
10	Barranca	Juliaca

Matching was employed to reduce imbalances in the randomization and was based on the city-specific STD prevalence found in the baseline survey, population size and type of city (coastal, Andean or jungle location). We believe that geographical separation of the cities will minimize contamination that could result from extensive sexual mixing between the randomization units. Because migration of sex workers or clients of FSW between cities could reduce the measured impact of the intervention, we are analyzing FSW migration before and during this trial.

The ten intervention cities are receiving a hybrid intervention, consisting of two key components: 1) training and support of pharmacy workers and referral networks of STD clinicians (RED PREVEN) for improved STD recognition and management and for STD/HIV prevention counseling; and

2) outreach to FSW to increase STD screening and treatment, and condom use. To supplement both intervention components, we also participated in a one-year social marketing campaign of condoms with the provision of STD treatment packets and promotion of condoms' double protection against STDs/HIV and unwanted pregnancy. The intervention began in July 2003 and will continue until April 2006, at which time the DSMB will recommend continuation or stopping the study.

2.1 Strengthened syndromic management of STIs[1][2][3]

STI syndromic management training. We began training of pharmacy workers and clinicians in private practice in the 10 intervention cities in July and August 2003, using a training protocol found to be efficacious and cost-effective in a district-randomized trial in Lima. The trainings were completed in mid-December 2003, and the RED PREVEN network with 564 certified clinicians and midwives, and 747 certified pharmacies with 2120 certified pharmacy workers was established. Simulated patients subsequently made 1800 visits to pharmacies in intervention and control cities to evaluate providers' syndromic management of urethral discharge, genital ulcers and vaginal discharge. Outcome measurements included whether or not they were offered appropriate treatment, suggested condom use and recommended partner treatment.

Informatics training & electronic dissemination of information. PREVEN Network members (pharmacy workers, midwives and physicians in private practice) received training in basic informatics to improve their computer capabilities and to learn how to use the RED PREVEN website (www.redpreven.org) to access additional resources. We initiated a CME credit course based on selected articles on STD/HIV prevention and management that were translated into Spanish. One article, a summary with comments, an editorial and a quiz on each article are sent monthly by mail to each of the members of the PREVEN Network. At the end of each cycle of 6 articles, physicians and midwives receive an equivalent of 1 CME credit through the Peruvian College of Physicians. Online case-scenario-based training was implemented in fall 2005 to continue and expand STI syndromic management training, allowing broader outreach and clinical updates.

Public awareness campaign. To complement the training of the health care professionals, we launched a public awareness campaign to inform men and women about the potential health risks of STDs, to raise

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[1] Garcia PJ, Holmes KK. Training Pharmacy workers in STD recognition, management, and prevention: A randomized control trial. WHO Bull 2003;81(11):806-814.

[2] Garcia PJ, Holmes KK. STD trends and patterns of treatment for STD by physicians in private practice in Peru. Sex Transm Infect. 2003 Oct;79(5):403-7.

[3] Adams EJ, Garcia PJ, Garnett GP, Edmunds WJ, Holmes KK. The cost-effectiveness of syndromic management in pharmacies in Lima, Peru. Sex Transm Dis. 2003 May;30(5):379-87.

awareness about STD symptom recognition and to promote the RED PREVEN network. An initial assessment and pilot testing resulted in the following key messages:

- Four out of 10 men and 5 out of 10 women have an STD without knowing it. STDs affect us all. Get information!
- (For men) "Pus from your penis or a burning sensation when you urinate is an STD. Get help from a RED PREVEN member"
- (For women) "Abnormal vaginal discharge - if you are sexually active and you have an increase in the amount vaginal discharge or a different odor or color, it could be an STD. Get help from a RED PREVEN member"
- RED PREVEN: We know about STDs. Look for the RED PREVEN sign in pharmacies, boticas and clinicians' offices.

Across the country, campaign activities included: promoting the key messages in the media, distributing merchandise and educational materials, and hosting educational workshops for parents, health workers and governmental authorities. As part of implementation efforts, we established formal collaboration agreements with key stakeholders in each city, which helped to form local STD task forces and community partnerships. For example, we partnered with a professional group of "mototaxistas" (a "mototaxi" is a small vehicle, similar to motorcycle, with a carriage on the back to carry 2-3 passengers). The drivers distributed STD prevention materials to their clients, which was particularly helpful in hard to reach areas. Pharmacy owners provided in-kind support by paying to have the materials reprinted. Media outlets in the different cities offered space in newspapers and programming time during radios and local TV broadcasts to present the campaign messages and conduct interviews of RED PREVEN members.

2.2 STD screening & treatment of female sex workers (FSW) at their worksites through a mobile outreach team[4]

Mobile teams, comprised of a health worker and a peer FSW educator in each of the 10 intervention cities, completed training in June 2003 and began visiting sex work venues in mid July 2003. Since that time, the mobile teams have visited each sex work venue every 8 weeks, which involves:

Collection of self-obtained vaginal swabs for detection of *N. gonorrhoeae*, *C. trachomatis* and *T. vaginalis*. Specimens are sent weekly on ice to the US NMRCD in Lima for GC/CT PCR testing, arrive frozen, are promptly tested, results are promptly returned by e-mail, and the mobile team travels back to the worksite one week after specimen collection to provide therapy for infected FSW.

[4] Sanchez J, Campos PE, Courtois B, Gutierrez L, Carrillo C, Alarcon J, Gotuzzo E, Hughes J, Watts D, Hillier SL, Buchanan K, Holmes KK. Prevention of sexually transmitted diseases (STDs) in female sex workers: prospective evaluation of condom promotion and strengthened STD services. Sex Transm Dis. 2003 Apr;30(4):273-9.

Presumptive treatment of trichomoniasis and bacterial vaginosis. Metronidazole 2 gm PO is given at each mobile team visit. For those who cannot avoid alcohol for 72 hours, treatment is provided the next week on the basis of InPouch cultures performed on the self-obtained vaginal swabs.

Promotion of consistent condom use and use of clinical services. Motivational interviewing (MI) techniques are used to promote correct and consistent condom use. FSW are also encouraged to visit their local public health clinic for routine screening and treatment. An additional mobile team member was added in each city in May 2005 to enhance counselling services.

Rapid syphilis serologic testing. In January 2005, we trained a laboratory technician in each of the 10 intervention cities to perform the Abbott Determine Syphilis TP assay, and implemented work-based rapid syphilis testing. FSW who test positive by Determine are referred to their local health clinic for treatment.

As new sex work venues are identified, they are added to the bimonthly outreach visits. The number of FSW screened every eight week cycle has increased from 1620 in cycle 1 to 2285 in cycle 14. Despite improving outreach to marginalized FSW, an average of 40% of FSW are seen for the first time during each cycle. We therefore now work with managers of commercial sex venues and with other FSW to rapidly refer new FSW to the mobile team.

2.3 One-year social marketing campaign of condoms & STI treatment packets

The goals of this supplementary intervention activity were to introduce into the 10 intervention cities a high quality, low price condom for the dual purpose of prevention of STI/HIV and prevention of unwanted pregnancies; and to introduce two treatment packages (Figure 3), one for urethral discharge in men (EFECTIPLUS, containing two 0.5 gm tablets of azithromycin and 1 500 mg tablet of ciprofloxacin) and one for vaginal discharge (EFECTIMAX, containing four 500 mg tablets of metronidazole) to facilitate the management of these common STI syndromes.

With funding from USAID Peru, the condom campaign was implemented from November 2003 - October 2004, in collaboration with the Peruvian NGO, APROPO. Distribution of the STI treatment packets began in May 2004 and will continue until the current production lot has been used. After the intervention trial ends, the plan is to introduce these packets commercially nationwide.

Figure 3. STD treatment packets

2.4 Prevention activities in control cities

In the control cities, there are no activities related to training pharmacists, pharmacy workers, nor physicians or midwives in private practice, in STD/HIV management.

The Ministry of Health's CERETS are special health centers that offer services to FSW in both intervention and control cities. These services include: physical examination, screening, treatment and counseling for STD/HIV. The screening provided is limited to wet mount for trichomonas, bacterial vaginosis and candida; gram stains of endocervical smears for gram negative diplococci (GC); and syphilis and HIV screening, contingent on availability of supplies. Condoms are offered free of charge. These clinics also offer Syndromic Management of STDs.

3. STUDY POPULATION

Outcome surveys in 2 populations are proposed:

1. Men and women from the general population; and
2. FSWs.

Note that the outcome surveys do not include a separate survey for clients of FSW. Our previous 2002 GPS found a high proportion (22%) of men reporting recent (<12 months) sex with FSW in the general population. STD prevalences in this subpopulation were similar to those found in the 2002 survey of clients. We believe that the sample of clients of FSW identified through the general population survey will provide useful data for our secondary objectives.

3.1 General Population Survey (GPS)

Men and women ages 18 to 29 years, residents of the 20 cities participating in the PREVEN study will be recruited for participation in this cross sectional study. The number of participants for each of the 20 cities will be 300 men and 300 women, totaling 12,000. Recruitment time will be three months.

Inclusion Criteria

Men and women who meet all of the following criteria are eligible for participation in this study:

- Men and women ages 18 to 29 years at enrollment
- Residents of the city where interview takes place, for a minimum of 6 months

- Lived in the city for at least 3 of the last 6 months
- Spend at least 4 nights a week in the household where identified
- Mentally capable of providing informed consent and to respond to a questionnaire
- Willing to participate in the survey by providing independent verbal informed consent

Participation in previous surveys, including our own STD surveys, does not preclude participation.

Exclusion Criteria

None

Participant Retention and Withdrawal

No follow-up of participants is required, and therefore no measures for retention are planned. Participants may voluntarily withdraw from the study for any reason at any time.

Recruitment Process

Training and Selection of Field Personnel

Field personnel will be trained in a nine-day course that will include modules on: Good Clinical Practice (GCP), ethics, basics of STDs, map handling, selection of participants, informed consent procedures, interviewing, sample collection and handling, and coding. The modules will include formal lectures as well as demonstrations and hands-on laboratory practice. All course participants will receive a copy of an Interviewer's Manual for the study. For each city, a team of approximately 3 interviewers and a supervisor will be selected. Trainees selected as supervisors will also receive a Supervisor's Manual. All interviewers and supervisors will be health professionals, such as nurses, midwives and medical doctors. Additionally, a team of 6 previously experienced regional coordinators and a national supervisor will be trained in this course. Trained field personnel, as well as supervisors and coordinators, will therefore be capable of conducting interviews.

Sampling Method

The sampling methods for this study are the same as those used in our 2002 baseline GPS. The sampling frame for the study will be based on 2005 data on household distribution from the Peruvian INEI. Clusters with an average size of 40 households will be defined by INEI. For each participating city, 108 clusters will be randomly selected.

Field workers will visit all households in the selected clusters, locating households with eligible members. A random sample of 10 households with eligible members will be selected from each cluster (or all households if less than 10).

The interviewers will return to the selected households. If more than one household member meets the inclusion criteria, the person with the most recent birthday will be selected for participation. At this time, an appointment to meet the selected person will be made. Selected participants will then be interviewed to confirm eligibility and to conduct the consent process to determine willingness to participate, and to complete the study procedures. Only in cases when eligibility of the participant could not be confirmed, the eligible person with the next most recent birthday will be selected.

Field Work

Field work will be divided into 9 periods lasting 10 days each, corresponding to the 9 independent samples selected for each city. Each interviewer has to cover 4 clusters during the first 8 days of each period. Although the ninth day of the period is programmed for visits to participants not found during the initial visit, there is no set limit for the number of appointments made to find selected participants. Recruitment of participants will continue until, at the end of a period, the gender-specific sample size has been reached or exceeded. It is expected that less than 100 clusters will be needed to reach the women's sample size, while in most cities, all 108 clusters will have to be visited to complete the sample size for men. Interviewers will conduct field procedures wearing study gowns and carrying a backpack with survey materials and a small portable cooler containing frozen acrylamide gel bags.

3.2 FSW Survey

For this study, FSW are defined as women who offer their personal sex services at venues of sex work identified in the participating cities. Two hundred FSWs will be enrolled for the study in each of the 20 cities, for a total of 4000 women. The recruitment period will be three months. Enrollment will be carried out in the same cities selected for the GPS.

Inclusion Criteria

FSWs who meet all of the following criteria will be eligible for inclusion in this study:

- Women found offering sex services at selected venues during pre-scheduled visits
- Age fourteen years or older
- Mentally capable of providing informed consent and responding to a questionnaire
- Willing to participate in the survey by providing independent verbal informed consent

Participation in previous surveys, including our own STD surveys, does not preclude participation.

Exclusion Criteria

FSWs unable to provide informed consent because of obvious mental impairment due to the use of alcohol or drugs will not be eligible for this study.

Participant Retention and Withdrawal

No follow-up of participants is required, and therefore no measures for retention are planned. Participants may voluntarily withdraw from the study for any reason at any time.

Recruitment Process

Training and Selection of Field Personnel

Local field work teams for each city will be composed of three counselors, a laboratory technician and a health promoter, usually a peer FSW. A centralized training workshop will be offered to counselors before the beginning of field activities. In addition to training in selection of study participants and the appropriate completion of the study questionnaire, the workshop will include modules on ethical issues including appropriate consent procedures granting confidentiality and avoiding any form of coercion. Laboratory technicians will receive training on appropriate techniques for sample collection, processing, and shipping, as well as on biosafety.

Sampling Method

In order to ensure comparability, sampling methods for this survey are similar to those used in our previous FSW survey. Local teams will begin field activities by performing a census of sex work venues (places where FSW meet their potential clients). These venues will be identified by the health promoter and key informants within each city. Team members will record the location and characteristics of the venues, as well as the approximate number of FSWs expected to be found at the venue at different times during an average week. The census will end when all accessible sex work venues have been recorded. Although it is expected that not all sex work venues will be identified by the census, extremely hard to reach venues are likely of low epidemiological importance, as they probably serve small closed circles of clients. At the end of the census, copies of the field work records will be sent to the UPCH in Lima. For cities where more than 200 FSWs were identified by the census, a sample of about 200 women will be selected by the time-location sampling method. For cities where fewer than 200 FSWs are identified, the local team will try to enroll all FSWs identified.

Local teams will visit commercial sex venues and invite all FSWs present in that venue to participate in the study. Sampling collection for this study will be completed in a single visit for all participants.

After providing verbal informed consent, study participants will complete an interviewer-administered questionnaire and undergo HIV and STD counseling and testing. All participants will receive a copy of the informed consent.

Basic demographic data will be collected from the FSWs who are present at the venue during the visit but who do not meet the eligibility criteria or who refuse to participate.

4. STUDY PROCEDURES – 2005 OUTCOME SURVEYS

4.1 General Population Survey

Interview

Participants will respond to a face-to-face questionnaire administered by the interviewer using a hand-held computer. Although the questionnaires to be used are roughly the same as the ones used in our 2002 surveys, a section on reproductive health has been added, and a few other minor changes have been introduced. The format of the questionnaire for the proposed survey will have to be adapted to

the hand-held computer, and pilot tested in the field. We believe that none of these changes will jeopardize the comparability of the two surveys.

The interviewer will demonstrate the use of the hand-held computer while administering the questionnaire. The questionnaire will collect data on demographic and household characteristics, travel, migration, labor, health seeking behavior, marriage, contraception, knowledge about STDs and reproductive health. Afterwards, the participants will respond to a self-administered questionnaire on the same hand-held computer, exploring characteristics of the participant's sexual behavior. This questionnaire will be completed privately by the participant. The computer program will restrict access to the responses given by the participants to the self-administered portion of the questionnaire.

Biological Sample Collection

After completing the questionnaires, the interviewers will explain the procedures for sample collection. Men will be asked to provide urine (about 15 ml) for STD tests in a 50-ml plastic container. Females will receive a rack with 1 polyester/plastic stick vaginal swab and two cotton/wooden stick vaginal swabs, which will be self-applied in that order by the participant. Then the interviewer will place the polyester swab in a 2 ml cryovial. The first cotton swab will be used to inoculate an InPouch TV® culture for *T. vaginalis* in the field. The second cotton swab will be used to prepare a vaginal smear in a glass slide in the field. Females unwilling to provide self-applied vaginal swabs will be asked to provide urine samples.

After collection of urine or vaginal samples the interviewers will collect venous blood samples from men and women using a vacuum extraction system. Syringes will be used when extraction of blood with the vacuum system is difficult.

Participants providing biological samples will receive a booklet with information about HIV and the HIV tests as pre-test counseling for HIV and a referral card to be used at a local health center to receive STD/HIV counseling and claim test results.

Urine samples, polyester swabs, and blood samples will be placed inside the cooler for transportation to the local specimen processing and transport laboratory facilities (SPTLF). *T. vaginalis* cultures and glass smears will be transported to the laboratory inside the backpack.

Consent forms, referral cards and samples will be labeled using preprinted self-adhesive labels with alphanumeric codes and barcodes. The same code will be entered into the hand-held computer.

Processing of Questionnaire Data

A first level validation of data will be programmed into the hand-held computer to prevent missing data and inconsistencies. Databases created by these computers will be encrypted, password-protected and transferred on a daily basis to a central server in Lima, where a second level validation will be carried out. Problems will be reported to local supervisors within 24 hours. Databases will then be transferred to STATA® or other appropriate data analysis software for analysis.

Processing of Biological Samples at the Local SPTLF

Samples collected by interviewers will be transported to the SPTLF within 4 hours of collection. At the SPTLF urine will be aliquoted into three 1.5-ml vials and frozen at –20° or less. *T. vaginalis* cultures will be placed at 37°C and examined under a microscope for the presence of the parasite every day for 5 days. Serum will be separated from blood and aliquoted into three 1.5-ml vials. All urine and sera aliquots, as well as the Polyester vaginal swabs, will be sent once a week to the central laboratory at Lima in shielded coolers. Our experience shows that samples shipped this way arrive to the central laboratory with temperatures below 3°C. All glass slides along with the *T. vaginalis* culture results will be sent to Lima once a month.

Processing at Central Laboratory

Temperature at the shielded coolers will be checked upon arrival to the central laboratory. All samples received will be inventoried with the aid of hand-held barcode readers and processed.

Urine samples and Polyester swabs will be used for the Cobas Amplicor® NG/CT PCR (Roche). All GC positive and a fraction of CT positive samples, as well as all samples with equivocal results will be sent to UW for confirmation. Vaginal smears will be Gram-stained and used for diagnosis of bacterial vaginosis using the Nugent's Score. Sera will be used for syphilis diagnosis using RPR nosticon II (Organon Teknika), with confirmation with Serodia- TPPA (Fujirebio, Inc.), and for the Uni-Form II Ag/Ab (Biomeieux-Vironostika) ELISA for HIV detection and NEW LAV BLOT I (BIO-RAD) as a confirmatory test for HIV infection. All diagnostic tests will be performed following manufacturers' procedures.

Return of Results to Survey Participants

All laboratory test results will be reported to the data manager, who will integrate them in a single database and generate individualized laboratory reports for the participants as shown in Figure 3. These reports will have the participant's code and will be sent to the counselors at the local health centers, where they will be available to the study participants. When indicated, treatment and counseling will be provided to participants and their partners at the local health centers. MINSA provides free counseling and treatment for curable STDs. The central laboratory in Lima performs several biological tests for a large number of samples (16,000 for the two surveys) collected over a relatively short period of time (3 months) in the 20 cities. Therefore, on top of the necessary delay in obtaining results due to the shipment of the samples to Lima, we expect a delay in the processing of samples, which will become more important towards the end of the surveys. We have therefore set a conservative period of 8 weeks between sample collection and the availability of STD test results to the participants. HIV screening test results will be distributed in a separate report, and will be available 4 weeks after blood collection.

All MINSA health centers are required to submit aggregated reports of STD cases seen by the institution. Additionally, individual anonymous reports are requested for every HIV infection diagnosed at health facilities. Counselors at local health centers will include cases detected by our study in their regular reports, in compliance with these regulations.

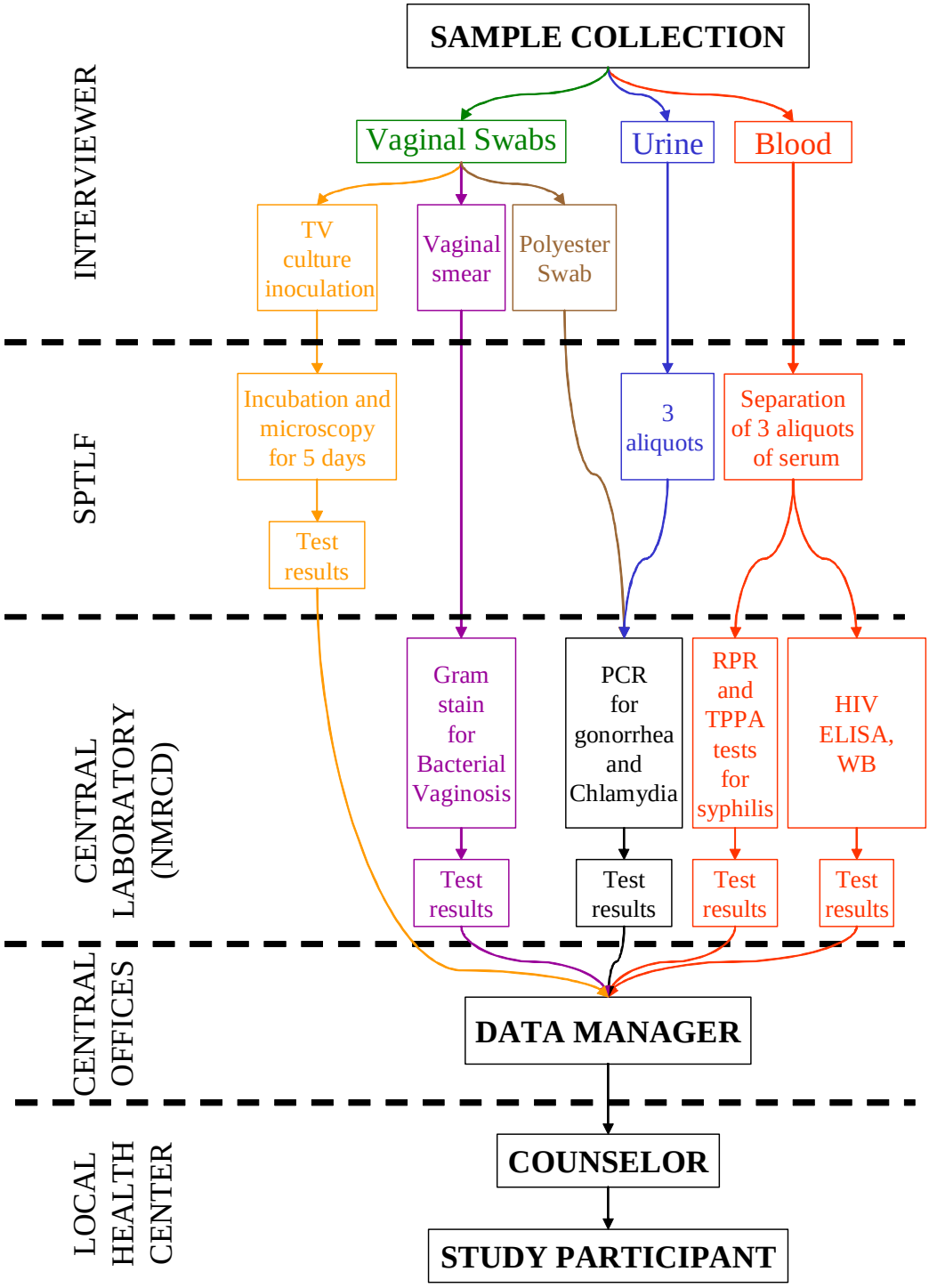


Figure 3. Flow of samples and laboratory test results GPS

4.2 FSW Survey

Interview

Detailed instructions to guide and standardize all study procedures across sites will be provided in the FSW Interviewers Manual of Procedures (MOP).

Anonymous, verbal informed consent for study participation and for the storage of blood for future testing will be obtained from all participants. When eligibility is not confirmed during the interview, the enrollment process will be aborted promptly. Next, the interviewer will administer the questionnaire.

Biological Sample Collection

Blood and vaginal swabs will be collected from consenting participants, following the same procedures described for the GPS in Section 4.1. Participants will receive a referral card to be used at the local health center to obtain their laboratory test results, and receive treatment if necessary. They will also be provided with condoms.

Processing of Questionnaire Data

The field team counselors will check questionnaires daily for completeness and consistency. On a weekly basis, reviewed questionnaires will be sent to the central data entry facility at Lima for transcription into databases. A second level validation will occur during data entry. Reports of problems will be returned to local teams within one week after receipt of questionnaires. Clean data will then be transferred to STATA® or other appropriate data analysis software for analysis.

Processing of Biological Samples

Blood samples and vaginal swabs will be processed following the same procedures described for the GPS in Section 4.1

Return of Results to Survey Participants

All laboratory test results will be reported to the data manager, who will integrate them in a single database, and generate individualized laboratory reports for the participants as described in Figure 4. These reports will have the participant's code and will be sent to the counselors at the local health centers, where they will be available to the study participants. When indicated, treatment and counseling will be provided to participants and their partners at the local health centers. The central laboratory in Lima performs several biological tests for a large number of samples (16,000 for the two surveys) collected over a relatively short period of time (3 months) in the 20 cities. Therefore, on top of the necessary delay in obtaining results due to the shipment of the samples to Lima, we expect a delay in the processing of samples, which will become more important towards the end of the surveys. We have therefore set a conservative period of 8 weeks between sample collection and the availability of STD test results to the participants. HIV screening test results will be distributed in a separate report, and will be available 4 weeks after blood collection.

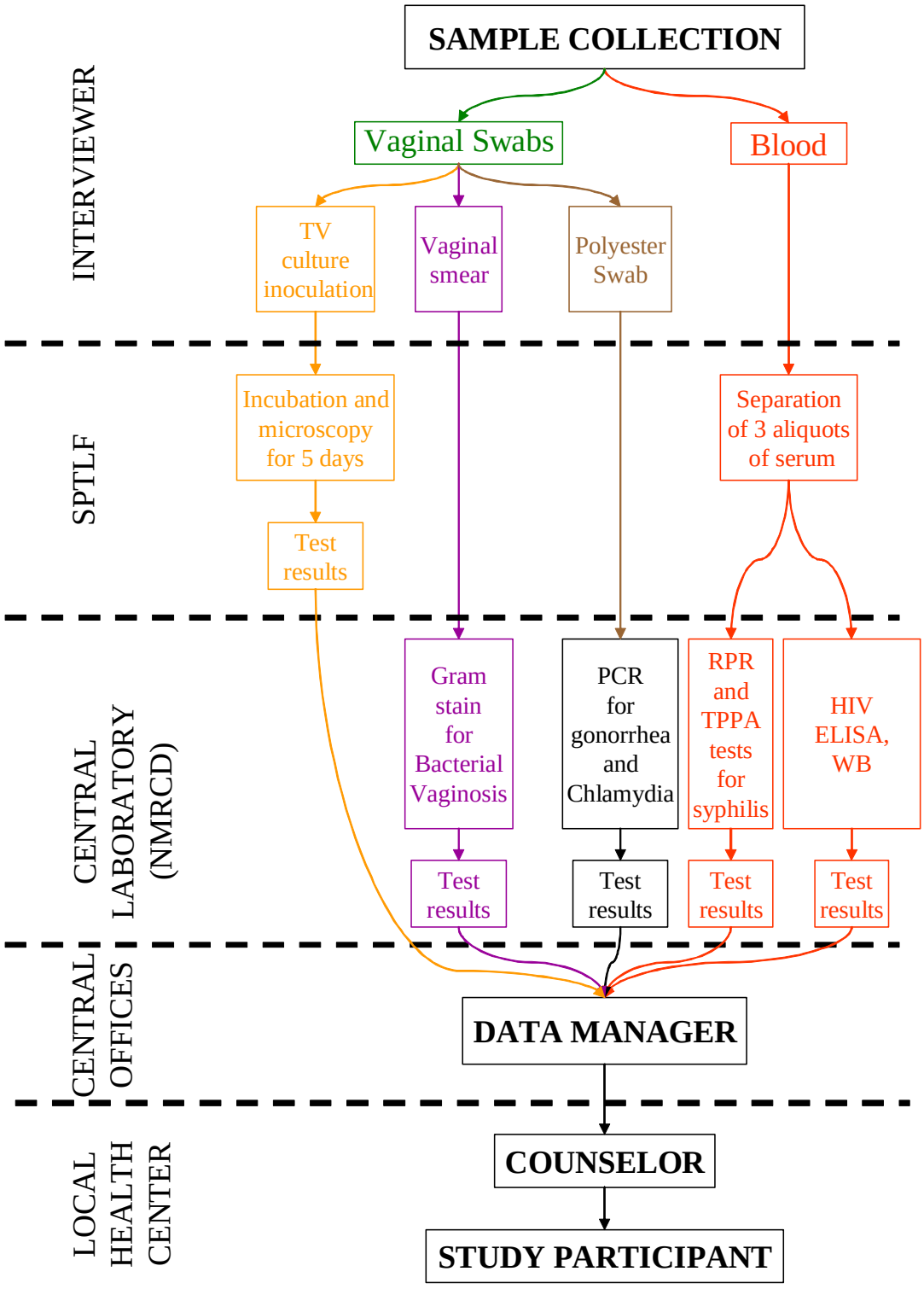


Figure 4. Flow of samples and laboratory test results FSW

5. POTENTIAL HARM TO PARTICIPANTS/ADVERSE EVENTS

Study participants may experience the following:

- Discomfort during phlebotomy. Participants may experience minor pain, bleeding and/or bruising at the venipuncture site, and/or vasovagal reactions. Some participants may experience lightheadedness.
- Embarrassment and concern when answering questions about risk factors of HIV and STD infection. Subjects may choose to not answer any question that makes them feel uncomfortable.
- Distress at finding out that they have HIV or another STD. All participants will receive pre-test and post-test counseling. See Section 7.5 for information about access to HIV-related care and for access to therapy for curable STDs.
- Discrimination if others learn participants' HIV status. To help protect human subjects, all study staff will receive training in safeguarding confidentiality as well as in the protection of human subjects. All information on participants will be kept extremely confidential and will not be shared with non-investigators.
- Other unforeseen potential harms: Although potential harm to participants in this study is expected to be minimal, the known risk will be thoroughly explained to participants as part of the informed consent process.

Although adverse events in this study are expected to be uncommon, the known risks will be thoroughly explained to participants as part of the informed consent process.

All interviewers, counselors, and laboratory technicians will record any unforeseen adverse event in their daily activity log and report it to their supervisors/coordinators. The supervisors/coordinators will generate electronic reports via the study's web page. The report will be accessible to the core team which will report it to the local IRB/EC.

6. STATISTICAL CONSIDERATIONS

6.1 Review of Study Design

This CRT is designed to test a multi-faceted intervention to reduce STDs. It will be conducted in 20 medium sized (> 50,000 people) cities in Peru. Ten cities will be randomized to the intervention and ten cities will serve as controls. The intervention consists of programs to educate pharmacy workers and health care providers, as well as outreach to FSWs. The primary endpoint will be a positive test for gonorrhea and/or chlamydia and/or trichomoniasis, and/or syphilis in a high risk group of FSW and in the lower risk young adult general population, as measured by the surveys described previously. The secondary endpoints that will be evaluated are positive tests for bacterial vaginosis and HIV. The study has power to determine if the intervention can reduce the burden of these STDs in the general population by 30-50% (depending on the STD). Changes of this magnitude have clear implications for public health. Smaller effects, even if present, may not be sufficient to change public health practice and, in any event, would be logistically very difficult to detect.

The surveys described above will be conducted in the 20 cities mentioned above over a period of 3 months. The proposed household based general population survey of men and women 18 to 29 years of age will be used to evaluate the study endpoints. In addition, we will conduct a venue-based survey of FSWs. Cluster sampling will be used for the former, while time-location sampling will be used for the latter survey.

6.2 Statistical testing of study endpoints

Power calculations

In Table 2 we present power calculations for testing for a 30-50% reduction in the primary STD outcomes between the treatment and control communities based on the random population survey of 600 men and women. The FSW population is considered separately. These calculations make use of the standard sample size/power formula for matched community randomized trials, as shown below.

$$n = 2 + \frac{(Z_{1-\alpha} + Z_{1-\beta})^2 \left[p_1(1-p_1)/f + p_2(1-p_2)/f + k^2(p_1^2 + p_2^2) \right]}{(p_1 - p_2)^2}$$

In this formula p_1 and p_2 are the disease prevalences in the treatment and control communities, respectively, *k* is the coefficient of variation of disease prevalence between communities, *f* is the number

of individuals per community and *n* is the number of communities. The Z's in the formula represent standard normal quantiles for appropriate type I and type II error rates. Disease prevalence in the control communities and the coefficient of variation are based on the baseline survey data. A paired t-test will be used to compare disease prevalence on the intervention and control cities.

Table 2. Power for comparing treatment (*n* = 10) and control communities (*n* = 10) given number per community, prevalence, α = 0.05 (1-tailed), coefficient of variation = .10 and indicated reduction in disease in treatment cities. Number of GPS results is reduced from 600 to 500 to allow for refusal to provide biologic samples.

	Number per community			Percent reduction		
	General population	FSW	Prev	30 Percent	40 Percent	50 Percent
Any STD	500		.10	.99	.99	.99
		200	.14	.87	.98	.99
Chlamydia	500		.04	.86	.98	.99
Trichomonas	250		.05	.72	.92	.99
Syphilis	500		.01	.41	.63	.81
HIV	500		.005	.27	.40	.57

6.3 Data Analysis

Primary Analyses

Primary Objective: *To evaluate the impact of a hybrid STD/HIV preventive intervention CRT on the prevalence of gonorrhea, chlamydia, trichomonas or syphilis at the population level.*

As randomization was done in a paired fashion (Section 6.1), a paired t-test will be used to compare disease prevalence for the intervention and control cities. This will be a very straight-forward analysis using the prevalence of the primary endpoint from the 20 Peruvian cities surveyed in the general population survey.

Secondary Analyses

Secondary Objective: *To evaluate the impact of a hybrid STD/HIV preventive intervention CRT on the prevalence of bacterial vaginosis and HIV infection.*

A paired t-test will be used to compare the intervention and control cities with respect to the secondary STD outcomes of gonorrhea, chlamydia, syphilis, trichomoniasis, HIV and bacterial vaginosis in the general population and the FSW.

PREVEN Trial DSMB Report to the Study Team

These notes document the meeting of the DSMB for the PREVEN Trial held at St. Mary's Campus, Imperial College, London on April 28th, 2006.

Participants

DSMB

Richard Hayes (Chair), Professor of Epidemiology and International Health, LSHTM
Sevgi Aral, Associate Director for Science, Centers for Disease Control & Prevention
Allan Donner, Professor of Epidemiology & Biostatistics, University of Western Ontario
David Mabey, Professor of Communicable Diseases, LSHTM
Miguel Campos, Professor of Mathematics, Universidad Peruana Cayetano Heredia

PREVEN Study Team

King Holmes, PI, University of Washington, Seattle
Patricia García, Co-PI, Universidad Peruana Cayetano Heredia
Geoff Garnett, Co-PI, Imperial College, London
César Cárcamo, General Population Surveys, Universidad Peruana Cayetano Heredia
Pablo Campos, FSW Intervention and Surveys, Universidad Peruana Cayetano Heredia
Peter White, Imperial College, London
James Hughes Statistician, University of Washington, Seattle
Anne Buffardi, University of Washington, Seattle

Presentations

Overview of Trial (King Holmes)
Randomization (Jim Hughes)
Baseline Surveys (César Cárcamo)
FSW Intervention and Process Outcomes (Pablo Campos)
Red PREVEN interventions and Process Outcomes (Patricia García)
Outcome Surveys (César Cárcamo)
Laboratory Issues (King Holmes)
Modeling (Geoff Garnett)
Stopping Guidelines and Format of Closed Report (Jim Hughes)

Documents

DSMB Charter, updated
PREVEN Presentation, updated Apr/8/2006
PREVEN Open Report, as of Apr/24/2006
PREVEN Closed Report, as of Apr/24/2006 (Restricted)
PREVEN Closed Report Addenda 1, Apr/25/2006 (Restricted)
PREVEN Closed Report Addenda 2, Apr/26/2006 (Restricted)
Closed Session Report of Teleconference held Mar/06/2006 (Restricted)

Open Session (from 9:00am to 12:30pm)

The following points were discussed in response to the presentations by the study team:

- Imbalance between treatment arms. It was noted that the unrestricted pair-matched randomization had resulted in a situation where baseline prevalences of the primary endpoint were generally higher in the intervention cities than their matched controls.
- Clarification of cluster classification. It was noted that in one of the matched pairs, the control city did not belong to the same geographical region as the corresponding intervention city, although STI prevalences were similar.
- Mobility of FSW. It was noted that mobility of the FSW was high, raising concerns over dilution of measured intervention effects. Mobility also poses challenges to delivery of the intervention, but these are likely to be realistic constraints, typical of many FSW settings.
- Inclusion of male sex workers (MSW). The study team reported that MSW are now being included in the intervention and that some evaluations are planned, although not as a formal part of the current trial.
- Prescription by pharmacists and self-medication. DSMB members asked if pharmacists are allowed to prescribe antibiotics in Peru. The team responded that, while this is not legally permitted, it is very common for pharmacists and non-pharmacist drug-store clerks to provide many kinds of drugs, antibiotics included, without prescription. DSMB members also asked about self-medication as could be practiced by FSW.
- Process evaluation of RED PREVEN intervention. It was noted that process measures for UD were generally better than for GU. The team commented that this may reflect long-standing public perceptions in Peru of UD as an STD, while this is less the case for GU.
- Changes in survey methods. The study team reported that survey methods had been modified for the outcome survey, particularly to improve the phrasing of questions and to implement PDA-based questionnaires. It was agreed that, while changes in methods during a trial should generally be avoided, in this case the benefits outweighed the risks. The primary analysis in this trial relates to comparisons between treatment arms at follow-up, rather than direct comparison of outcome versus baseline measurements.
- Changes in sampling frame. It was noted that the sampling frame for the GPS, maintained by the Peruvian statistical agency (INEI), was based on a census carried out >10y earlier which, although continuously updated at block level, may not be accurate. It was reported that a new census was carried out in 2005, but the data were not available for the study to use.
- FSW sampling frames. It was agreed that there may be some differences between arms in the quality of sampling frames for FSW. These were based on updated lists of venues which may be more complete in intervention cities. The study team reported that they made extra efforts to update lists in control cities, but conceded that this type of bias could not be ruled out.
- Laboratory issues. It was reported by the study team that GC tests used had less than desired sensitivity as compared with newer PCR tests. Confirmed results are being used for the analysis. It was also confirmed that TV testing was undertaken locally by MoH technicians in each city, and this limited the scope for standardization and quality control by the study team.
- Data updates. An inconsistency in Table 11 of the open report was noted. The study team reported that some of the tables and figures had been amended following recent laboratory and data processing, and may not fully match the open and closed report figures.

Feedback to Study Team (from 2:00pm to 2:30pm)

The DSMB congratulated the study team on the conduct of the study, which appears to have been carried out to a very high standard.

Guided by the stopping guidelines, and after reviewing all the data provided by the study team in the open and closed reports, the DSMB had unanimously agreed to recommend continuation of the trial for a third year of intervention, with final follow-up surveys towards the end of 2006.

The following specific comments and recommendations were provided to the study team:

1) Adverse Events. No SAEs were reported. Reported adverse events were mostly attributable to the side-effects of metronidazole which are well-known. The DSMB suggested that the study team consider monitoring for any *non-biological* adverse events that might be associated with the intervention, such as medication practices, behavior changes or stigmatization.

2) Data Quality. The DSMB were very impressed by the high coverage rates achieved in the follow-up survey. The concerns raised about GC testing are already being addressed by the study team. The DSMB recommended that the team should review the sampling frames used for the GPS, and make an effort to validate these against the new INEI census data that are now available.

3) Adherence to the Protocol. The DSMB commended the study team on its efficient handling of all violations reported. The DSMB supported the inclusion of FSW aged under18 years for both intervention activities and evaluation surveys, and also favored their inclusion in the data analysis. This should be formally recorded as a *protocol amendment.* The DSMB was pleased to note that decisive and appropriate measures had been put in place to address a small number of violations related to staff misconduct, and noted that the rate of staff problems was quite low for this type of study.

4) External Evidence. DSMB members were not aware of any external factors or other study results which would affect the appropriateness of continuing the PREVEN trial. Members emphasized the uniqueness of this trial and the importance of completing it successfully.

5) FSW Mobility. The DSMB noted that the high mobility of FSW may introduce contamination leading to dilution of the measured intervention effect, and recommended that every effort should be made (in the FSW survey and during intervention rounds) to record rates and geographical locations of migration to help interpret trial results.

6) Rate of Incomplete Testing. The DSMB noted that the proportion of participants with incomplete laboratory data was higher in the control arm than the intervention arm. The study team was advised to investigate possible reasons for imbalance, which could potentially lead to selection bias, and to consider ways of minimizing this during the final follow-up.

7) Trichomoniasis. The DSMB reported their concerns about the validity of the TV data due to outlying values in some cities, very low correlation between baseline and follow-up TV rates, a low matching correlation, and the lack of standardization in this endpoint noted by the study team. Since TV rates were relatively high compared with other STIs, there was concern that *noise* in this endpoint could unduly influence the trial results. The DSMB therefore recommended that the primary endpoint for the trial should be amended by omitting TV from the composite STI variable. Sample size calculations should be rechecked to confirm that the trial is still adequately powered for the amended endpoint, although this is expected to be the case. Despite this recommendation, the DSMB considered that intervention effects on TV were also of intrinsic interest. The study team should investigate whether TV testing could be repeated or confirmed in a central location, using standardized procedures. If so, separate analysis of TV findings could be carried out in addition to analysis of the primary endpoint.

8) Matched Analysis. Based on information given in the closed report and the strong matching correlation achieved for the amended primary endpoint, the DSMB recommended that the matched pairs should be retained in the final data analysis.

9) Chlamydia. Noting that some women provided urine while some provided vaginal swabs for CT testing, the DSMB recommended that the combined prevalence (based on *either urine or vaginal swab*) be used in the primary analysis. A secondary analysis (in women) should be performed based on vaginal swabs alone, providing take-up rates of swabs were similar in the two treatment arms.

10) Analytical Plan. The DSMB recommended that the study team should prepare an analytical plan in advance of breaking the code for the final analysis. The imbalance between treatment arms in baseline STI rates will require an *adjusted analysis* to be performed. It will also be important to carry out gender-specific analyses for each endpoint (while bearing in mind the problem of multiple comparisons). The DSMB would be willing to provide comments on a draft analytical plan.

11) Additional Studies. The study provides an important opportunity to carry out additional studies taking advantage of the research setting and the high participation rates. Two lines for such studies are noted here. Cost-effectiveness studies, based on cost and effectiveness data from the trial and mathematical modeling, would be very helpful to support policy-making. It would also be very important to measure behavioral outcomes in the trial as accurately as possible to assist interpretation of the trial results, whether these are positive or negative. It is likely that both qualitative and quantitative methods of data collection will be needed, allowing for triangulation of different methods. The very high coverage achieved in this trial suggests a highly compliant study population, and the study team should consider the possible implications of this for social desirability bias.

12) For completeness, the DSMB also requested the amended analysis for FSW data based on the amended primary endpoint (omitting TV).

Finally, while its functions formally terminated at the end of this meeting, the DSMB expressed the willingness of all members to provide further assistance to the project in future, if this would be helpful.

PREVEN Trial Data Analysis Plan

Title: PREVEN Community Randomized Trial – A hybrid HIV/STD intervention on a high-risk group of female sex workers (FSW) and on the lower-risk young adult general population.

Background and Overview:
The primary aim of this trial is to evaluate the impact of a hybrid STD/HIV prevention intervention on the prevalence of sexually transmitted infections (STIs) (including gonorrhea, chlamydia, trichomonas, syphilis and HIV) in female sex workers (FSW) and the young adult general population (GP). The study is designed as a prospective community randomized trial of 20 medium sized cities in Peru. The intervention was begun in 2003 and the primary outcome (STI prevalence) will be measured in two cross sectional surveys (one in a population of FSW and the other in the general population of young adults) in 2006 in each of the 20 Peruvian cities. The surveys will also provide us with estimates of the fraction of HIV cases that are diagnosed and treated in existing programs (Ministry of Health, Army, Navy, Police health services, Social Security system and other programs in Peru), the prevalence of HIV viremia, the prevalence of bacterial vaginosis (BV) and risk behaviors. In secondary analyses we will use this information to identify characteristics of bridging populations and determining the structure of sexual networks. This information will be directly useful for planning nationwide programs that integrate prevention and treatment.

Broad objective: To evaluate the impact of a hybrid STD/HIV preventive intervention CRT on the prevalence of sexually transmitted infections (STIs), gonorrhea, chlamydia, trichomonas, and syphilis, in a high-risk group of female sex workers (FSW) and in the lower risk young adult general population.

Specific aims:
Primary

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the combined prevalence of gonorrhea and/or chlamydia and/or syphilis (RPR $\geq$ 1:8) and/or trichomonas (in women) in the general population of young adults.

Secondary

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the combined prevalence of gonorrhea and/or chlamydia and/or syphilis (RPR $\geq$ 1:8, TP•PA confirmed) and/or trichonomas in female sex workers.
- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalences of bacterial vaginosis and HIV in the general population of young adults.
- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalences of bacterial vaginosis and HIV on female sex workers.
- To evaluate the impact of a hybrid STD/HIV prevention intervention on condom use in the GP and FSW
- To determine if the effectiveness of the intervention differs in GP individuals who prefer to use pharmacies for treatment of STI-related symptoms
- To determine if the effectiveness of the intervention differs in GP men who use FSW

Study Design:
Overview: The study is a community randomized trial of 20 medium sized cities in Peru. The cities were initially paired based on STI prevalence measured in a baseline (pre-intervention) survey. One member of each pair was then randomized to receive the intervention, which consists of two primary components: i) pharmacist education to strengthen syndromic management of STI, and ii) outreach to female sex workers (FSW) using a mobile team. The primary outcome (STI prevalence) will be measured in two cross

sectional surveys - one among FSW and the other in the general population of young adults - in each of the 20 Peruvian cities after the intervention has been in place for approximately 2 years.

Baseline Surveys: Baseline surveys of STI prevalence in the general population (18-29 year old men and women), FSW and their clients were conducted in 24 midsize (> 50,000) cities in Peru in 2002 and 2003. These surveys collected both behavioral information and biologic samples.

The general population survey (GPS) used year 2000 census data to randomly select households by cluster sampling (each cluster was an average of 40 households). A random sample of clusters was selected for each city. Then a census was conducted for each household in the selected clusters and a random sample of households with eligible members was selected. Within each selected household, one eligible individual (male or female, aged 18 - 29, living in the city for at least 6 months) was randomly selected.

The FSW/client surveys used time-venue sampling. In each city the study team mapped known locations of FSW. Each day of the week was then divided into x parts and a random sample of time/venues was selected. All (?) sex workers present at the chosen venue at the chosen time were invited to participate in the survey.

Randomization: From the 24 cities that took part in the 2002 general population and 2003 FSW and client surveys, twenty were selected to participate in the CRT. Matching was employed to reduce imbalances in the randomization. Matching was based primarily on total STI prevalence (chlamydia, trichomoniasis, syphilis, gonorrhea, HIV) in the baseline general population survey. Population size and location (coastal, highland, jungle) were used as secondary matching criteria. Ten cities (one member of each matched pair) were then randomized to receive the intervention. Table 2 shows the matched pairs.

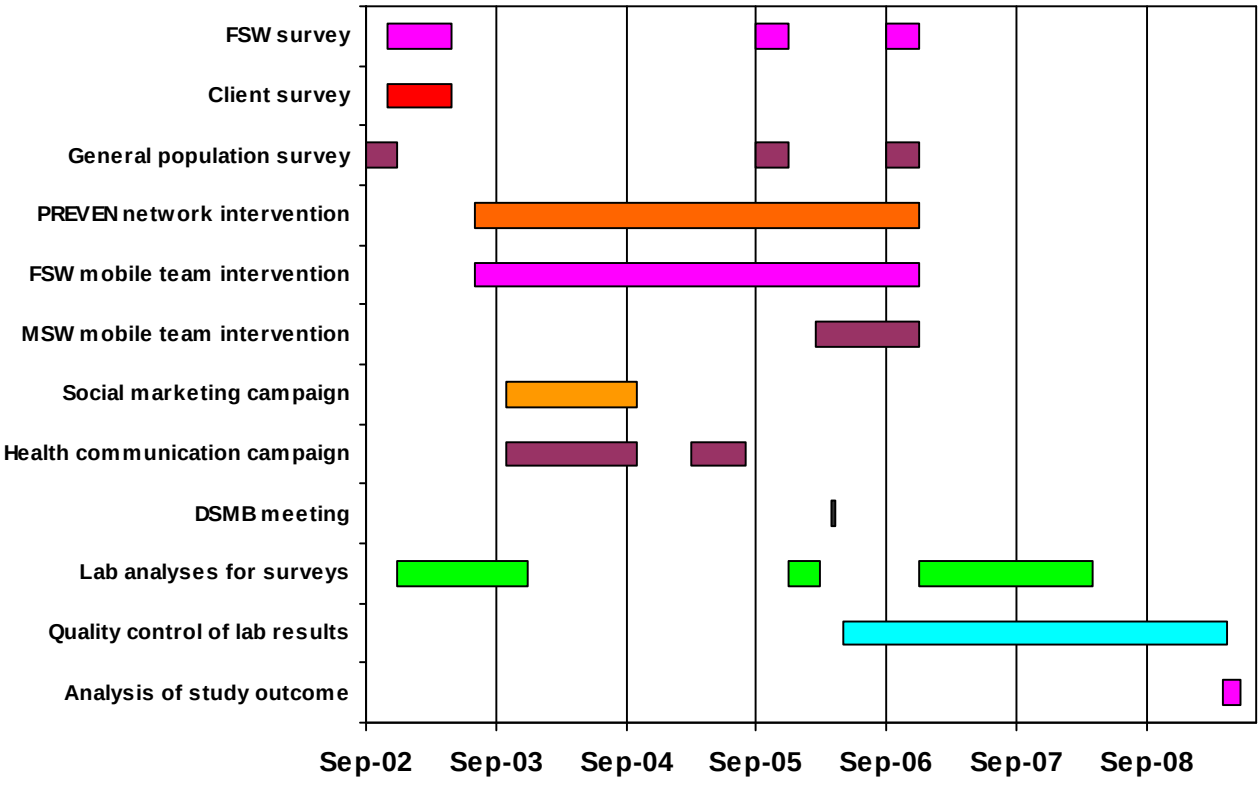
Table 2. Matched city pairs for the PREVEN project

	Matched pairs	
1	Chimbote	Piura
2	Ilo	Tumbes
3	Ayacucho	Cajamarca
4	Tacna	Ica
5	Huaraz	Cerro de Pasco
6	Huancayo	Cuzco
7	Talara	Chincha
8	Tarapoto	Huanuco
9	Iquitos	Pucallpa
10	Barranca	Juliaca

Intervention: The intervention consists of two key components: 1) training and support of pharmacy workers and referral networks of STD clinicians for improved STI recognition and management and for STD/HIV prevention counseling (RED PREVEN component); and 2) outreach to FSW via mobile teams (consisting of a health worker and a peer FSW educator) to increase STI screening and treatment, and condom use (FSW component). The intervention began in July 2003 and will continue through (at least) the final outcome surveys (December 2006). To supplement both intervention components, the study also included a one-year (October 2003 – October 2004) social marketing campaign of condoms with the provision of STD treatment packets and promotion of condoms' double protection against STDs/HIV and unwanted pregnancy.

Outcome Surveys: Interim surveys of the GP and FSW were conducted in 2005 and final outcome surveys will be conducted in 2006. The format and content of the surveys will be similar to the baseline surveys described above although the questionnaire data will be collected using a handheld (Palm) computer.

The figure below shows the timeline of the various components of the study.



Testable hypotheses: The testable hypotheses for this study will have the form

$$\text{Ho: } p_{control} = p_{intervention}$$
$$\text{Ha: } p_{control} \neq p_{intervention}$$

where p_i is the prevalence of the outcome (combined or individual STIs) in the i'th group. The section on inferential analysis below contains details on how this hypothesis will be tested.

Variables:

The following table lists variables that will be used in the statistical analyses.

Variable	**Definition**	**How collected**	**Units**	**Use in analysis**
pcrcturine	**CT in urine by PCR**	**GPS**	**0 = negative; 1 = positive; 2 = indeterminate**	**Outcome component**
pcrctswab	**CT in vaginal swab by PCR**	**GPS**	**0 / 1 / 2**	**Outcome component**
Ctresul	Prevalence of Chlamydia infection **by PCR**	Urine samples tested by Cobas (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5= indeterminate) in 2006, with description: "chlamydia trachomatis cobas result". Indeterminates treated as missing.** **GPS: Created variable for 2006 such that: vaginal swab results used where swab provided; otherwise urine result used. For men all samples were urine. Indeterminate results treated as missing.**	0 - 1	Outcome
Pcrgcurine	**GC in urine by PCR**	**GPS**	**0 / 1 / 2 (2= indeterminate)**	**Outcome component**
Pcrgcswab	**GC in vag. swab by PCR**	**GPS**	**0 / 1 / 2**	**Outcome component**
Genprgcuri	**GC in urine by PCR (Genprobe)**	**GPS**	**0 / 1 / 2**	**Outcome component**
Genprgcswa	**GC in swab by PCR (Genprobe)**	**GPS**	**0 / 1 / 2**	**Outcome component**
taqmangcur	**Taqman GC inurine**	**GPS - received variable in 2006 but contained no data – will we use this?**	**?**	**Didn't use**
taqmangcsw	**Taqman GC in swab**	**"**	**?**	**Didn't use**
Ngresul	Prevalence of gonorrhea infection **by PCR**	Urine samples tested by Cobas (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5=**	0 - 1	Outcome

		indeterminate) in 2006 with description: "neisseria gonorrhoea cobas result confirmed by aptima". Indeterminates treated as missing. **GPS: Created variable from other variables received in 2006 so that: Vaginal swab results used where women provided swab; otherwise result from urine sample used. For men urines used. We required positives to be confirmed by Genprobe. Indeterminates and positives without available confirmation results were treated as missing.**		
NewSyph	Prevalence of **elevated serum titer indicating recent** syphilis infection	Blood samples tested by RPR **and confirmed by TPPA**; result of each test is positive (titer $\geq$ 1:8) or negative (titer < 1:8) and individual results are aggregated to the city level***.** **GPS and FSW: Created variable in 2006 to accomplish this, using rpr, rpr dilution and tppa results.**	0 - 1	Outcome
HIVresul	Prevalence of HIV infection	Blood sample tested by dual ELISA and confirmed by WB; result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5= indeterminate) in 2006 with description: "hiv result". Indeterminates treated as missing.** **GPS: created variable to match above description, from separate wb and elisa results.**	0 - 1	Outcome
Tvresul	Prevalence of trichomonas infection	Urine samples tested by InPouch culture (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* (female only)	0 - 1	Outcome
BV	Prevalence of bacterial vaginosis	??; result of each test is positive or negative and individual results are aggregated to the city level* (female only)	0 - 1	Outcome
CtGcSy	Prevalence of	**GPS and FSW:** Created from	0 - 1	Primary

	Chlamydia and/or gonorrhea and/or syphilis infection	ctresult, gcresul, newsyph and **aggregated to** city level* **If any of the 3 test results was missing, combined outcome treated as missing.**		outcome
City	City code	assigned	1 - 20	
Arm	Randomization arm	assigned	0 = control 1 = intervention	Primary predictor
Pair	Randomization pair	assigned	1 - 10	Stratification variable
Age	Age (yrs)	GPS FF Q1.2		Covariate
Gender	Gender	GPS FF Q1.1	1=male 2=female	Covariate
Read	Can you read?	GPS FF Q1.4	1=yes 2=no 9=no response	
Married/Cohab	Marital status	GPS FF Q7.1	1=Single 2=Married 3=Cohabiting 4=Separated 5=Divorced 6=Widow/er 9=No response	
Eversex	Ever had sex:	GPS SA Q1.11 (female) Q1.12 (male)	0=no/1=yes	
Sexfsw	Sex w/prostitute:	GPS SA Q2.6 (males only, who replied 'yes' to 'eversex'),	0=no/1=yes	
Sexfswnum	How many times sex w/prostitute in last 12 months:	GPS SA Q2.9 (males only, who replied 'yes' to 'sexfsw')	0=None; 1-10=actual #; 11=more than 10; 66=doesn't remember	
Sexfswnocondom	How many times sex w/ prostitute w/o condom in last 12 months	GPS SA Q2.10 (males who gave response of 1 or more to 'sexfswnocondom');	1-4=actual #; 5=more than 4; 6=Always uses condom; 66=Doesn't remember	
Nocondc	Noncondom use w/ prostitute in last 12 months (men only):	Created variable Omission in code creating variable for interim analysis caused rates to be dramatically overestimated; See note.	1=yes (had sex with prostitute without condom in last year); 0=no (always used condom or no sex with prostitute)	Outcome

Sexmsm	Sex with male	GPS SA Q2.11 (males only)		
Msmnum	How many times sex w/male	GPS SA Q2.14 (males only, but variable "msmnum" received at interim analysis shows everyone responding to this including women and men who did not acknowledge MSM. Looks like not correct data for this question.)	0=None 1-4=? 66=Doesn't remember	
Msmnocond	How many times sex w/male w/o condom	GPS SA Q2.15 (males only, and those who acknowledge MSM.)	1-4=actual #; 5= more than 4; 6=Always uses condom; 66=Doesn't remember	
Nocondm	Noncondom use with male in last 12 months (men only)	Created variable Omission in code creating variable for interim analysis was an oversight and led to overstating rates; see note.	1=yes (had sex with male without condom in last year); 0=no (always used condom or no sex with male)	Outcome
Sexlastpartnum	Number of times sex with last partner in last 3 months	GPS SA Q3.11	1-20=actual # 21=more than 20; 66=doesn't remember	
Sexlastpartnocondom	Number of times sex with last partner in last 3 months without condom	GPS SA Q3.12	1-20=actual # 21=more than 20; 22=always uses condom; 66=doesn't remember	
Nocondl	Noncondom use w/last partner (GPS)	Created variable	1=yes (had sex with last sex partner without condom in last year); 0=no (always used condom or no sex in last 3 months)	Outcome
hlthseek	Health seeking behavior – "If you had abnormal secretion … what would you do first?"	GPS SA Q2.1	=Nothing =Consult friend =Pharmacy =Doc/Clinic =Hospital =Meds @ home =Other	
Workplace	Workplace type for	FSW questionnaire Q503 (last 7 days)	1=Brothel;	covariate

	FSW	or Q504 (last month)	2=Nightclub; 3=Bar; 4=Street; Other 99=no response	
Culinter	Condom use w/ last intercourse (FSW)	FSW questionnaire Q618 (is this only among women whose last sex was vaginal intercourse? – Q617?)	1=Yes 2=No (I created 'cl', same variable but 0=no/1=yes)	Outcome
	Can read/write?	FSW questionnaire – Q204	1=yes to both 2=can read 3=can write 4=neither	covariate
	Ever married or cohabitant?	FSW questionnaire – Q304	1=yes, 2=no, 99=no response	covariate
	Living with sex partner?	FSW questionnaire – Q309. For FSW, Will make 0/1 variable (1,2,4=yes, 3 or 5=no)	1=married live w/spouse; 2=married live with other sex ptr 3=married not living with sex ptr 4=not married, cohab; 5 = not married not cohab 99=no response	covariate
	Have dependents?	FSW questionnaire – Q310	1=yes, 2=no, 99=no response	Covariate
	Charged last client	FSW questionnaire – Q616 if last sex partner was client. If second to last sex partner was last client, then Q629. If sex partner before that, then Q643		

* To aggregate results to the city level the number of positive results in a given city/survey are divided by the number of nonmissing results from that city/survey.

Statistical Analysis:

Descriptive analyses

The following tables will be presented:

- Baseline general population survey (GPS) process outcomes by city (refusal rate, sample size, % giving blood, urine, swabs)[Cesar has done]
- Baseline STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) in the GPS by city [Cesar has done]

- 2006 GPS process outcomes by city (refusal rate, sample size, % giving blood, urine, swabs) [Cesar]
- 2006 GPS demographics (age, gender, etc) by city [UW]
- 2006 GPS STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) by city [UW]
- 2006 GPS rates of condom use among men with commercial sex partners in the last year, rates of condom use among men with male sex partners in the last year; rates of condom use among men and women with their last partner in the last 3 months; by gender [UW]

- Baseline female sex worker (FSW) survey process outcomes by city (refusal rate, sample size, % giving specimens) [Pablo has done]
- Baseline STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) in the FSW survey by city [Pablo has done]
- 2006 FSW survey process outcomes by city (refusal rate, sample size, % giving specimens) [Pablo]
- 2006 FSW demographics (age, workplace type, etc) by city [UW]
- 2006 FSW survey STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) by city [UW]
- 2006 FSW rates of condom use at the last sexual encounter; by city; by intervention arm [UW]

The following graph will also be presented:

- FSW STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) over time in the interventions cities, pooled [Pablo]

Inferential analyses

Primary analysis

The unit of analysis will be the city (i.e. STI prevalence at the city level). This approach is appropriate since the sample size is approximately the same from city to city. The primary analysis of intervention efficacy will be based on the model

$$d_i^O = b_0 + b_1 d_i^B + \varepsilon_i \qquad \text{(M1)}$$

where d_i^O is the difference in the primary STI endpoint (combined gonorrhea, chlamydia, trich in women, and syphilis prevalence) between the intervention and control communities (control – intervention) of the *i*'th pair in the final (2006) outcome survey, d_i^B is the corresponding difference in the baseline survey and ε_i is a random error. The hypothesis of interest is

Ho: $b_0 = 0$	(no intervention effect)	(H1)
Ha: $\beta_0 \neq 0$	(intervention effect)	

Note that if d_i^B was not included in the model, this analysis would be equivalent to a paired t-test. The d_i^B term is included in the model to control for differences in STI prevalence at baseline (which may occur in spite of the matching).

Least squares regression will be used for model fitting. The standardized test statistic $\hat{b}_0 / se(\hat{b}_0)$ from this analysis has a t-distribution with 8 degrees of freedom. A p-value will be reported for the hypothesis (H1) and a type I error rate (α) of 0.05 (two-tailed) will be used to reject/fail to reject the null hypothesis (H1).

The endpoint in the primary analysis will be **combsti** as measured in the GP.

Secondary analysis

The analysis outlined above will also be used for the following endpoints:

- **combsti** in FSW
- **Ctresul, gcresul, newsyph, tvresul, HIVresul, BV** in GPS
- **Ctresul, gcresul, newsyph, tvresul, HIVresul, BV** in FSW
- **Nocondl** in GPS
- **Culinter** in FSW

To determine if the intervention is more effective in particular subsets of individuals (i.e. those who prefer to use pharmacies for treatment of STI-related symptoms) we will use an individual level analysis of the 2006 GPS data based on the following model

$$\text{logit}(p_{ij}) = b_0 + b_1 T_i + b_2 B_i + b_3 X_{ij} + b_4 T_i X_{ij} + a_i$$

where T_i is a 0/1 treatment indicator for city i, B_i is the baseline disease prevalence for city i, X_{ij} is a 0/1 covariate indicating whether subject j in city i belongs to the subset of interest (i.e. uses FSW) and a_i is a random city effect ($a_i \sim N(0,\sigma^2)$). The outcome in this analysis will be **combsti.** In the absence of the interaction term, the b_1 coefficient from this model estimates the overall treatment effect (comparable to b_0 in model H1). With the interaction term in the model, the hypothesis Ho: $b_4 = 0$ will be used to test if the intervention effect on combsti differs across levels of X (e.g. we might expect b_4 to be positive – intervention more effective – in individuals who prefer to use pharmacies for treatment of STI-related symptoms). A two-sided 0.05 level test will be used.

- To determine if the effectiveness of the intervention differs in individuals who prefer to use pharmacies for STI-related symptoms, X_{ij} = **hlthseek**.
- To determine if the effectiveness of the intervention differs in men who use FSW, X_{ij} = **sexfsw** and the analysis is restricted to men

PREVEN Trial DSMB Report to the Study Team

These notes document the meeting of the DSMB for the PREVEN Trial held at St. Mary's Campus, Imperial College, London on April 28th, 2006.

Participants

DSMB

Richard Hayes (Chair), Professor of Epidemiology and International Health, LSHTM
Sevgi Aral, Associate Director for Science, Centers for Disease Control & Prevention
Allan Donner, Professor of Epidemiology & Biostatistics, University of Western Ontario
David Mabey, Professor of Communicable Diseases, LSHTM
Miguel Campos, Professor of Mathematics, Universidad Peruana Cayetano Heredia

PREVEN Study Team

King Holmes, PI, University of Washington, Seattle
Patricia García, Co-PI, Universidad Peruana Cayetano Heredia
Geoff Garnett, Co-PI, Imperial College, London
César Cárcamo, General Population Surveys, Universidad Peruana Cayetano Heredia
Pablo Campos, FSW Intervention and Surveys, Universidad Peruana Cayetano Heredia
Peter White, Imperial College, London
James Hughes Statistician, University of Washington, Seattle
Anne Buffardi, University of Washington, Seattle

Presentations

Overview of Trial (King Holmes)
Randomization (Jim Hughes)
Baseline Surveys (César Cárcamo)
FSW Intervention and Process Outcomes (Pablo Campos)
Red PREVEN interventions and Process Outcomes (Patricia García)
Outcome Surveys (César Cárcamo)
Laboratory Issues (King Holmes)
Modeling (Geoff Garnett)
Stopping Guidelines and Format of Closed Report (Jim Hughes)

Documents

DSMB Charter, updated
PREVEN Presentation, updated Apr/8/2006
PREVEN Open Report, as of Apr/24/2006
PREVEN Closed Report, as of Apr/24/2006 (Restricted)
PREVEN Closed Report Addenda 1, Apr/25/2006 (Restricted)
PREVEN Closed Report Addenda 2, Apr/26/2006 (Restricted)
Closed Session Report of Teleconference held Mar/06/2006 (Restricted)

Open Session (from 9:00am to 12:30pm)

The following points were discussed in response to the presentations by the study team:

- Imbalance between treatment arms. It was noted that the unrestricted pair-matched randomization had resulted in a situation where baseline prevalences of the primary endpoint were generally higher in the intervention cities than their matched controls.
- Clarification of cluster classification. It was noted that in one of the matched pairs, the control city did not belong to the same geographical region as the corresponding intervention city, although STI prevalences were similar.
- Mobility of FSW. It was noted that mobility of the FSW was high, raising concerns over dilution of measured intervention effects. Mobility also poses challenges to delivery of the intervention, but these are likely to be realistic constraints, typical of many FSW settings.
- Inclusion of male sex workers (MSW). The study team reported that MSW are now being included in the intervention and that some evaluations are planned, although not as a formal part of the current trial.
- Prescription by pharmacists and self-medication. DSMB members asked if pharmacists are allowed to prescribe antibiotics in Peru. The team responded that, while this is not legally permitted, it is very common for pharmacists and non-pharmacist drug-store clerks to provide many kinds of drugs, antibiotics included, without prescription. DSMB members also asked about self-medication as could be practiced by FSW.
- Process evaluation of RED PREVEN intervention. It was noted that process measures for UD were generally better than for GU. The team commented that this may reflect long-standing public perceptions in Peru of UD as an STD, while this is less the case for GU.
- Changes in survey methods. The study team reported that survey methods had been modified for the outcome survey, particularly to improve the phrasing of questions and to implement PDA-based questionnaires. It was agreed that, while changes in methods during a trial should generally be avoided, in this case the benefits outweighed the risks. The primary analysis in this trial relates to comparisons between treatment arms at follow-up, rather than direct comparison of outcome versus baseline measurements.
- Changes in sampling frame. It was noted that the sampling frame for the GPS, maintained by the Peruvian statistical agency (INEI), was based on a census carried out >10y earlier which, although continuously updated at block level, may not be accurate. It was reported that a new census was carried out in 2005, but the data were not available for the study to use.
- FSW sampling frames. It was agreed that there may be some differences between arms in the quality of sampling frames for FSW. These were based on updated lists of venues which may be more complete in intervention cities. The study team reported that they made extra efforts to update lists in control cities, but conceded that this type of bias could not be ruled out.
- Laboratory issues. It was reported by the study team that GC tests used had less than desired sensitivity as compared with newer PCR tests. Confirmed results are being used for the analysis. It was also confirmed that TV testing was undertaken locally by MoH technicians in each city, and this limited the scope for standardization and quality control by the study team.
- Data updates. An inconsistency in Table 11 of the open report was noted. The study team reported that some of the tables and figures had been amended following recent laboratory and data processing, and may not fully match the open and closed report figures.

Feedback to Study Team (from 2:00pm to 2:30pm)

The DSMB congratulated the study team on the conduct of the study, which appears to have been carried out to a very high standard.

Guided by the stopping guidelines, and after reviewing all the data provided by the study team in the open and closed reports, the DSMB had unanimously agreed to recommend continuation of the trial for a third year of intervention, with final follow-up surveys towards the end of 2006.

The following specific comments and recommendations were provided to the study team:

1) Adverse Events. No SAEs were reported. Reported adverse events were mostly attributable to the side-effects of metronidazole which are well-known. The DSMB suggested that the study team consider monitoring for any *non-biological* adverse events that might be associated with the intervention, such as medication practices, behavior changes or stigmatization.

2) Data Quality. The DSMB were very impressed by the high coverage rates achieved in the follow-up survey. The concerns raised about GC testing are already being addressed by the study team. The DSMB recommended that the team should review the sampling frames used for the GPS, and make an effort to validate these against the new INEI census data that are now available.

3) Adherence to the Protocol. The DSMB commended the study team on its efficient handling of all violations reported. The DSMB supported the inclusion of FSW aged under18 years for both intervention activities and evaluation surveys, and also favored their inclusion in the data analysis. This should be formally recorded as a *protocol amendment.* The DSMB was pleased to note that decisive and appropriate measures had been put in place to address a small number of violations related to staff misconduct, and noted that the rate of staff problems was quite low for this type of study.

4) External Evidence. DSMB members were not aware of any external factors or other study results which would affect the appropriateness of continuing the PREVEN trial. Members emphasized the uniqueness of this trial and the importance of completing it successfully.

5) FSW Mobility. The DSMB noted that the high mobility of FSW may introduce contamination leading to dilution of the measured intervention effect, and recommended that every effort should be made (in the FSW survey and during intervention rounds) to record rates and geographical locations of migration to help interpret trial results.

6) Rate of Incomplete Testing. The DSMB noted that the proportion of participants with incomplete laboratory data was higher in the control arm than the intervention arm. The study team was advised to investigate possible reasons for imbalance, which could potentially lead to selection bias, and to consider ways of minimizing this during the final follow-up.

7) Trichomoniasis. The DSMB reported their concerns about the validity of the TV data due to outlying values in some cities, very low correlation between baseline and follow-up TV rates, a low matching correlation, and the lack of standardization in this endpoint noted by the study team. Since TV rates were relatively high compared with other STIs, there was concern that *noise* in this endpoint could unduly influence the trial results. The DSMB therefore recommended that the primary endpoint for the trial should be amended by omitting TV from the composite STI variable. Sample size calculations should be rechecked to confirm that the trial is still adequately powered for the amended endpoint, although this is expected to be the case. Despite this recommendation, the DSMB considered that intervention effects on TV were also of intrinsic interest. The study team should investigate whether TV testing could be repeated or confirmed in a central location, using standardized procedures. If so, separate analysis of TV findings could be carried out in addition to analysis of the primary endpoint.

8) Matched Analysis. Based on information given in the closed report and the strong matching correlation achieved for the amended primary endpoint, the DSMB recommended that the matched pairs should be retained in the final data analysis.

9) Chlamydia. Noting that some women provided urine while some provided vaginal swabs for CT testing, the DSMB recommended that the combined prevalence (based on *either urine or vaginal swab*) be used in the primary analysis. A secondary analysis (in women) should be performed based on vaginal swabs alone, providing take-up rates of swabs were similar in the two treatment arms.

10) Analytical Plan. The DSMB recommended that the study team should prepare an analytical plan in advance of breaking the code for the final analysis. The imbalance between treatment arms in baseline STI rates will require an *adjusted analysis* to be performed. It will also be important to carry out gender-specific analyses for each endpoint (while bearing in mind the problem of multiple comparisons). The DSMB would be willing to provide comments on a draft analytical plan.

11) Additional Studies. The study provides an important opportunity to carry out additional studies taking advantage of the research setting and the high participation rates. Two lines for such studies are noted here. Cost-effectiveness studies, based on cost and effectiveness data from the trial and mathematical modeling, would be very helpful to support policy-making. It would also be very important to measure behavioral outcomes in the trial as accurately as possible to assist interpretation of the trial results, whether these are positive or negative. It is likely that both qualitative and quantitative methods of data collection will be needed, allowing for triangulation of different methods. The very high coverage achieved in this trial suggests a highly compliant study population, and the study team should consider the possible implications of this for social desirability bias.

12) For completeness, the DSMB also requested the amended analysis for FSW data based on the amended primary endpoint (omitting TV).

Finally, while its functions formally terminated at the end of this meeting, the DSMB expressed the willingness of all members to provide further assistance to the project in future, if this would be helpful.

PREVEN Trial Data Analysis Plan

Title: PREVEN Community Randomized Trial – A hybrid HIV/STD intervention on a high-risk group of female sex workers (FSW) and on the lower-risk young adult general population.

Background and Overview:
The primary aim of this trial is to evaluate the impact of a hybrid STD/HIV prevention intervention on the prevalence of sexually transmitted infections (STIs) (including gonorrhea, chlamydia, trichomonas, syphilis and HIV) in female sex workers (FSW) and the young adult general population (GP). The study is designed as a prospective community randomized trial of 20 medium sized cities in Peru. The intervention was begun in 2003 and the primary outcome (STI prevalence) will be measured in two cross sectional surveys (one in a population of FSW and the other in the general population of young adults) in 2006 in each of the 20 Peruvian cities. The surveys will also provide us with estimates of the fraction of HIV cases that are diagnosed and treated in existing programs (Ministry of Health, Army, Navy, Police health services, Social Security system and other programs in Peru), the prevalence of HIV viremia, the prevalence of bacterial vaginosis (BV) and risk behaviors. In secondary analyses we will use this information to identify characteristics of bridging populations and determining the structure of sexual networks. This information will be directly useful for planning nationwide programs that integrate prevention and treatment.

Broad objective: To evaluate the impact of a hybrid STD/HIV preventive intervention CRT on the prevalence of sexually transmitted infections (STIs), gonorrhea, chlamydia, trichomonas, and syphilis, in a high-risk group of female sex workers (FSW) and in the lower risk young adult general population.

Specific aims:
Primary

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the combined prevalence of gonorrhea and/or chlamydia and/or syphilis (RPR $\geq$ 1:8) in the general population of young adults.

Secondary

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalences of trichomonas, bacterial vaginosis and HIV in the general population of young adults.
- To evaluate the impact of a hybrid STD/HIV preventive intervention on the combined prevalence of gonorrhea and/or chlamydia and/or syphilis (RPR $\geq$ 1:8) on female sex workers.
- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalences of trichomonas, bacterial vaginosis and HIV on female sex workers.
- To evaluate the impact of a hybrid STD/HIV prevention intervention on condom use in the GP and FSW
- To determine if the effectiveness of the intervention differs in GP individuals who prefer to use pharmacies for treatment of STI-related symptoms
- To determine if the effectiveness of the intervention differs in GP men who use FSW

Study Design:
Overview: The study is a community randomized trial of 20 medium sized cities in Peru. The cities were initially paired based on STI prevalence measured in a baseline (pre-intervention) survey. One member of each pair was then randomized to receive the intervention, which consists

of two primary components: i) pharmacist education to strengthen syndromic management of STI, and ii) outreach to female sex workers (FSW) using a mobile team. The primary outcome (STI prevalence) will be measured in two cross sectional surveys - one among FSW and the other in the general population of young adults - in each of the 20 Peruvian cities after the intervention has been in place for approximately 2 years.

Baseline Surveys: Baseline surveys of STI prevalence in the general population (18-29 year old men and women), FSW and their clients were conducted in 24 midsize (> 50,000) cities in Peru in 2002 and 2003. These surveys collected both behavioral information and biologic samples.

The general population survey (GPS) used year 2000 census data to randomly select households by cluster sampling (each cluster was an average of 40 households). A random sample of clusters was selected for each city. Then a census was conducted for each household in the selected clusters and a random sample of households with eligible members was selected. Within each selected household, one eligible individual (male or female, aged 18 - 29, living in the city for at least 6 months) was randomly selected.

The FSW/client surveys used time-venue sampling. In each city the study team mapped known locations of FSW. Each day of the week was then divided into x parts and a random sample of time/venues was selected. All (?) sex workers present at the chosen venue at the chosen time were invited to participate in the survey.

Randomization: From the 24 cities that took part in the 2002 general population and 2003 FSW and client surveys, twenty were selected to participate in the CRT. Matching was employed to reduce imbalances in the randomization. Matching was based primarily on total STI prevalence (chlamydia, trichomoniasis, syphilis, gonorrhea, HIV) in the baseline general population survey. Population size and location (coastal, highland, jungle) were used as secondary matching criteria. Ten cities (one member of each matched pair) were then randomized to receive the intervention. Table 2 shows the matched pairs.

Table 2. Matched city pairs for the PREVEN project

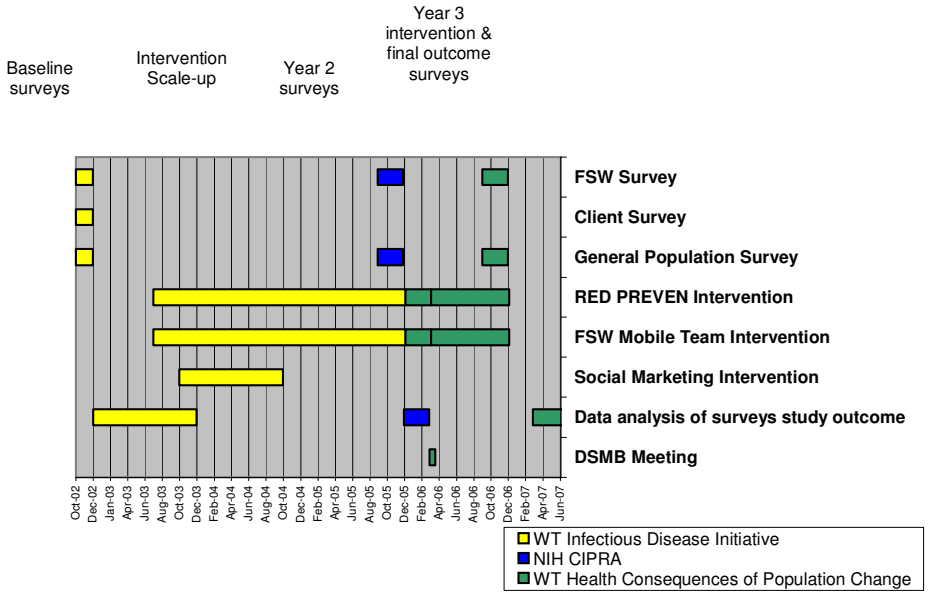
	Matched pairs	
1	Chimbote	Piura
2	Ilo	Tumbes
3	Ayacucho	Cajamarca
4	Tacna	Ica
5	Huaraz	Cerro de Pasco
6	Huancayo	Cuzco
7	Talara	Chincha
8	Tarapoto	Huanuco
9	Iquitos	Pucallpa
10	Barranca	Juliaca

Intervention: The intervention consists of two key components: 1) training and support of pharmacy workers and referral networks of STD clinicians for improved STI recognition and management and for STD/HIV prevention counseling (RED PREVEN component); and 2) outreach to FSW via mobile teams (consisting of a health worker and a peer FSW educator) to increase STI screening and treatment, and condom use (FSW component). The intervention

began in July 2003 and will continue through (at least) the final outcome surveys (December 2006). To supplement both intervention components, the study also included a one-year (October 2003 – October 2004) social marketing campaign of condoms with the provision of STD treatment packets and promotion of condoms' double protection against STDs/HIV and unwanted pregnancy.

Outcome Surveys: Interim surveys of the GP and FSW were conducted in 2005 and final outcome surveys will be conducted in 2006. The format and content of the surveys will be similar to the baseline surveys described above although the questionnaire data will be collected using a handheld (Palm) computer.

The figure below shows the timeline of the various components of the study.



Testable hypotheses: The testable hypotheses for this study will have the form

$$\text{Ho: } p_{control} = p_{intervention}$$
$$\text{Ha: } p_{control} \neq p_{intervention}$$

where p_i is the prevalence of the outcome (combined or individual STIs) in the i'th group. The section on inferential analysis below contains details on how this hypothesis will be tested.

Variables:

The following table lists variables that will be used in the statistical analyses.

Variable	Definition	How collected	Units	Use in analysis
pcrcturine	**CT in urine by PCR**	**GPS**	**0 = negative; 1 = positive; 2 = indeterminate**	**Outcome component**
pcrctswab	**CT in vaginal swab by PCR**	**GPS**	**0 / 1 / 2**	**Outcome component**
Ctresul	Prevalence of Chlamydia infection **by PCR**	Urine samples tested by Cobas (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5= indeterminate) in 2006, with description: "chlamydia trachomatis cobas result". Indeterminates treated as missing.** **GPS: Created variable for 2006 such that: vaginal swab results used where swab provided; otherwise urine result used. For men all samples were urine. Indeterminate results treated as missing.**	0 - 1	Outcome
Pcrgcurine	**GC in urine by PCR**	**GPS**	**0 / 1 / 2 (2= indeterminate)**	**Outcon compor**
Pcrgcswab	**GC in vag. swab by PCR**	**GPS**	**0 / 1 / 2**	**Outcon compor**
Genprgcuri	**GC in urine by PCR (Genprobe)**	**GPS**	**0 / 1 / 2**	**Outcon compor**
Genprgcswa	**GC in swab by PCR (Genprobe)**	**GPS**	**0 / 1 / 2**	**Outcon component**
taqmangcur	**Taqman GC inurine**	**GPS - received variable in 2006 but contained no data – will we use this?**	**?**	**Didn't use**
taqmangcsw	**Taqman GC in swab**	**"**	**?**	**Didn't use**
Ngresul	Prevalence of gonorrhea infection **by PCR**	Urine samples tested by Cobas (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5=**	0 - 1	Outcome

Comment [CFASTD1]:
(in stata):

```
/* make CT variable */

gen ctresul=.
replace ctresul=pcrcturine if
gender==1
replace ctresul=pcrctswab if
gender==0    /* if female use swab
result where available. (some men
have them; ignore) */
replace ctresul=pcrcturine if
gender==0 & pcrctswab==.
label value ctresul posneg
```

		indeterminate) in 2006 with description: "neisseria gonorrhoea cobas result confirmed by aptima". Indeterminates treated as missing. **GPS: Created variable from other variables received in 2006 so that: Vaginal swab results used where women provided swab; otherwise result from urine sample used. For men urines used. We required positives to be confirmed by Genprobe. Indeterminates and positives without available confirmation results were treated as missing.**		
NewSyph	Prevalence of **elevated serum titer indicating recent** syphilis infection	Blood samples tested by RPR **and confirmed by TPPA**; result of each test is positive (titer ≥ 1:8) or negative (titer < 1:8) and individual results are aggregated to the city level***.** **GPS and FSW: Created variable in 2006 to accomplish this, using rpr, rpr dilution and tppa results.**	0 - 1	Outcom
HIVresul	Prevalence of HIV infection	Blood sample tested by dual ELISA and confirmed by WB; result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5= indeterminate) in 2006 with description: "hiv result". Indeterminates treated as missing.** **GPS: created variable to match above description, from separate wb and elisa results.**	0 - 1	Outcom
Tvresul	Prevalence of trichomonas infection	Urine samples tested by InPouch culture (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* (female only)	0 - 1	Outcome
BV	Prevalence of bacterial vaginosis	??; result of each test is positive or negative and individual results are aggregated to the city level* (female only)	0 - 1	Outcome
CtGcSy	Prevalence of	**GPS and FSW:** Created from	0 - 1	Primary

Comment [CFASTD2]:
In stata:

```
/* make GC variable */

gen ngurine=pcrgcurine
replace ngurine=0 if genprgcuri==0
/* only keep positives if confirmed
by genprobe */
replace ngurine=.a if
(pcrgcurine==1 & genprgcuri==.)
/* make missing if confirmation not
done. */
label value ngurine posneg

gen ngswab=pcrgcswab
replace ngswab=0 if genprgcswa==0
replace ngswab=.a if (pcrgcswab==1
& genprgcswa==.)
label value ngswab posneg

gen ngresul=.
replace ngresul=ngurine if
gender==1
replace ngresul=ngswab if gender==0
/* if female use swab result where
available. (some men have them;
ignore) */
replace ngresul=ngurine if
gender==0 & ngresul==.
label value ngresul posneg
```

	Chlamydia and/or gonorrhea and/or syphilis infection	ctresult, gcresul, newsyph and **aggregated to** city level* **If any of the 3 test results was missing, combined outcome treated as missing.**		outcome
City	City code	assigned	1 - 20	
Arm	Randomization arm	assigned	0 = control 1 = intervention	Primary predictor
Pair	Randomization pair	assigned	1 - 10	Stratification variable
Age	Age (yrs)	GPS FF Q1.2		Covariate
Gender	Gender	GPS FF Q1.1	1=male 2=female	Covariate
Read	Can you read?	GPS FF Q1.4	1=yes 2=no 9=no response	
Married/Cohab	Marital status	GPS FF Q7.1	1=Single 2=Married 3=Cohabiting 4=Separated 5=Divorced 6=Widow/er 9=No response	
Eversex	Ever had sex:	GPS SA Q1.11 (female) Q1.12 (male)	0=no/1=yes	
Sexfsw	Sex w/prostitute:	GPS SA Q2.6 (males only, who replied 'yes' to 'eversex'),	0=no/1=yes	
Sexfswnum	How many times sex w/prostitute in last 12 months:	GPS SA Q2.9 (males only, who replied 'yes' to 'sexfsw')	0=None; 1-10=actual #; 11=more than 10; 66=doesn't remember	
Sexfswnocondom	How many times sex w/ prostitute w/o condom in last 12 months	GPS SA Q2.10 (males who gave response of 1 or more to 'sexfswnocondom');	1-4=actual #; 5=more than 4; 6=Always uses condom; 66=Doesn't remember	
Nocondc	Noncondom use w/ prostitute in last 12 months (men only):	Created variable Omission in code creating variable for interim analysis caused rates to be dramatically overestimated; See note.	1=yes (had sex with prostitute without condom in last year); 0=no (always used condom or no sex with prostitute)	Outcom

Comment [CFASTD3]:
(in stata Code in italics added 1/9/2007; omission was an oversight.):

```
gen nocondc=1-(sexfswnocondom==6)
replace nocondc=.a if sexfswnocondom==66
replace nocondc=. If sexfswnocondom=.
replace nocondc=0 if sexfswnum==0 & gender==1
replace nocondc=0 if sexfsw==0 & gender==1
replace nocondc=0 if eversex==0 & gender==1
replace nocondc=. if gender==0 | gender==.
```

Omission in code creating variable caused rates to be dramatically overestimated for interim analysis; those who hadn't had sex w/FSW in last 12 months (but who had previously) were mistakenly assigned 1 rather than 0 (of 1407 assigned 1, these accounted for 941).

Sexmsm	Sex with male	GPS SA Q2.11 (males only)		
Msmnum	How many times sex w/male	GPS SA Q2.14 (males only, but variable "msmnum" received at interim analysis shows everyone responding to this including women and men who did not acknowledge MSM. Looks like not correct data for this question.)	0=None 1-4=? 66=Doesn't remember	
Msmnocond	How many times sex w/male w/o condom	GPS SA Q2.15 (males only, and those who acknowledge MSM.)	1-4=actual #; 5= more than 4; 6=Always uses condom; 66=Doesn't remember	
Nocondm	Noncondom use with male in last 12 months (men only)	Created variable Omission in code creating variable for interim analysis was an oversight and led to overstating rates; see note.	1=yes (had sex with male without condom in last year); 0=no (always used condom or no sex with male)	Outcom
Sexlastpartnum	Number of times sex with last partner in last 3 months	GPS SA Q3.11	1-20=actual # 21=more than 20; 66=doesn't remember	
Sexlastpartnocondom	Number of times sex with last partner in last 3 months without condom	GPS SA Q3.12	1-20=actual # 21=more than 20; 22=always uses condom; 66=doesn't remember	
Nocondl	Noncondom use w/last partner (GPS)	Created variable	1=yes (had sex with last sex partner without condom in last year); 0=no (always used condom or no sex in last 3 months)	Outcom
hlthseek	Health seeking behavior – "If you had abnormal secretion … what would you do first?"	GPS SA Q2.1	=Nothing =Consult friend =Pharmacy =Doc/Clinic =Hospital =Meds @ home =Other	
Workplace	Workplace type for	FSW questionnaire Q503 (last 7 days)	1=Brothel;	covariate

Comment [CFASTD4]:

(in stata: Code in italics added 1/9/2007; omission was an oversight.):

```
gen nocondm=1-(msmnocondom==6)
replace nocondm=.a if msmnocondom==66
replace nocondm=. if msmnocondom==.
replace nocondm=0 if msmnum==0 & gender==1
replace nocondm=0 if msm==0 & gender==1
replace nocondm=0 if eversex==0 & gender==1
replace nocondm=. if gender==0 | gender==.
```

Omission for interim analysis was an oversight and led to overstating rates (all MSM got labeled as 1, should not have).

Comment [CFASTD5]:

(in stata: Code in italics corrected1/9/2007; omission was an oversight.):

```
gen nocondl=1-(sexlastpartnocondom==22*)
replace nocondl=.a if sexlastpartnocondom==66
replace nocondl=. if sexlastpartnocondom==. /* didn't change variable */
replace nocondl=0 if sexlastpartnum==0
replace nocondl=0 if eversex==0
```

For the interim analysis, I mistakenly assumed that the code for always using condom was 6, as for the 2 previous condom use variables. It should have been 22. Thus 115 people were assigned 0 when they should have been 1 and 1314 were assigned 1 when should have been 0, so nocondom use in last 3 months with last sex partner were overestimated.

	FSW	or Q504 (last month)	2=Nightclub; 3=Bar; 4=Street; Other 99=no response	
Culinter	Condom use w/ last intercourse (FSW)	FSW questionnaire Q618 (is this only among women whose last sex was vaginal intercourse? – Q617?)	1=Yes 2=No (I created 'cl', same variable but 0=no/1=yes)	Outcome
	Can read/write?	FSW questionnaire – Q204	1=yes to both 2=can read 3=can write 4=neither	covariate
	Ever married or cohabitant?	FSW questionnaire – Q304	1=yes, 2=no, 99=no response	covariate
	Living with sex partner?	FSW questionnaire – Q309. For FSW, Will make 0/1 variable (1,2,4=yes, 3 or 5=no)	1=married live w/spouse; 2=married live with other sex ptr 3=married not living with sex ptr 4=not married, cohab; 5 = not married not cohab 99=no response	covariate
	Have dependents?	FSW questionnaire – Q310	1=yes, 2=no, 99=no response	Covariate
	Charged last client	FSW questionnaire – Q616 if last sex partner was client. If second to last sex partner was last client, then Q629. If sex partner before that, then Q643		

* To aggregate results to the city level the number of positive results in a given city/survey are divided by the number of nonmissing results from that city/survey.

Statistical Analysis:

Descriptive analyses

The following tables will be presented:

- Baseline general population survey (GPS) process outcomes by city (refusal rate, sample size, % giving blood, urine, swabs)[Cesar has done]

- Baseline STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) in the GPS by city [Cesar has done]
- 2006 GPS process outcomes by city (refusal rate, sample size, % giving blood, urine, swabs) [Cesar]
- 2006 GPS demographics (age, gender, etc) by city [UW]
- 2006 GPS STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) by city [UW]
- 2006 GPS rates of condom use among men with commercial sex partners in the last year, rates of condom use among men with male sex partners in the last year; rates of condom use among men and women with their last partner in the last 3 months; by gender [UW]

- Baseline female sex worker (FSW) survey process outcomes by city (refusal rate, sample size, % giving specimens) [Pablo has done]
- Baseline STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) in the FSW survey by city [Pablo has done]
- 2006 FSW survey process outcomes by city (refusal rate, sample size, % giving specimens) [Pablo]
- 2006 FSW demographics (age, workplace type, etc) by city [UW]
- 2006 FSW survey STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) by city [UW]
- 2006 FSW rates of condom use at the last sexual encounter; by city; by intervention arm [UW]

The following graph will also be presented:

- FSW STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) over time in the interventions cities, pooled [Pablo]

Inferential analyses

Primary analysis

The unit of analysis will be the city (i.e. STI prevalence at the city level). This approach is appropriate since the sample size is approximately the same from city to city. The primary analysis of intervention efficacy will be based on the model

$$d_i^O = b_0 + b_1 d_i^B + \varepsilon_i \qquad \text{(M1)}$$

where d_i^O is the difference in the primary STI endpoint (combined gonorrhea, chlamydia and syphilis prevalence) between the intervention and control communities (control – intervention) of the *i*'th pair in the final (2006) outcome survey, d_i^B is the corresponding difference in the baseline survey and ε_i is a random error. The hypothesis of interest is

Ho: $b_0 = 0$ (no intervention effect) (H1)
Ha: $\beta_0 \neq 0$ (intervention effect)

Note that if d_i^B was not included in the model, this analysis would be equivalent to a paired t-test. The d_i^B term is included in the model to control for differences in STI prevalence at baseline (which may occur in spite of the matching).

Least squares regression will be used for model fitting. The standardized test statistic $\hat{b}_0 / se(\hat{b}_0)$ from this analysis has a t-distribution with 8 degrees of freedom. A p-value will be reported for the hypothesis (H1) and a type I error rate (α) of 0.05 (two-tailed) will be used to reject/fail to reject the null hypothesis (H1).

The endpoint in the primary analysis will be **ctgcsy** as measured in the GP.

Secondary analysis

The analysis outlined above will also be used for the following endpoints:

- **Ctgcsy** in FSW
- **Ctresul, gcresul, newsyph, tvresul, HIVresul, BV** in GPS
- **Ctresul, gcresul, newsyph, tvresul, HIVresul, BV** in FSW
- **Nocondl** in GPS
- **Culinter** in FSW

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To determine if the intervention is more effective in particular subsets of individuals (i.e. those who prefer to use pharmacies for treatment of STI-related symptoms) we will use an individual level analysis of the 2006 GPS data based on the following model

$$\text{logit}(p_{ij}) = b_0 + b_1 T_i + b_2 B_i + b_3 X_{ij} + b_4 T_i X_{ij} + a_i$$

where T_i is a 0/1 treatment indicator for city i, B_i is the baseline disease prevalence for city i, X_{ij} is a 0/1 covariate indicating whether subject j in city i belongs to the subset of interest (i.e. uses FSW) and a_i is a random city effect ($a_i \sim N(0,\sigma^2)$). The outcome in this analysis will be **ctgcsy.** In the absence of the interaction term, the b_1 coefficient from this model estimates the overall treatment effect (comparable to b_0 in model H1). With the interaction term in the model, the hypothesis Ho: $b_4 = 0$ will be used to test if the intervention effect on ctgcsy differs across levels of X (e.g. we might expect b_4 to be positive – intervention more effective – in individuals who prefer to use pharmacies for treatment of STI-related symptoms). A two-sided 0.05 level test will be used.

- To determine if the effectiveness of the intervention differs in individuals who prefer to use pharmacies for STI-related symptoms, X_{ij} = **hlthseek**.
- To determine if the effectiveness of the intervention differs in men who use FSW, X_{ij} = **sexfsw** and the analysis is restricted to men

Dummy Tables:

General Population Survey (GPS) Process Outcomes

City	Invited to participate	Refusal rate	Provided questionnaire data, n(%)	Provided blood, n(%)	Provided Genital Samples		
					Males	Females	
					Urine, n(%)	Urine, n(%)	Vaginal swabs, n(%)
Ayacucho							
Barranca							
Cajamarca							
Chimbote							
Chincha							
Cusco							
Huancayo							
Huanuco							
Huaraz							
Ica							
Ilo							
Iquitos							
Juliaca							
Pasco							
Piura							
Pucallpa							
Tacna							
Talara							
Tarapoto							
Tumbes							
Intervention cities							
Control cities							
Total							

GPS Demographic Data

City	Age mean (range)	% male	<3 years in this city	Can read/ write?	Ever had sex?	Ever married/ cohab?	Living w/ sex partner
Ayacucho							
Barranca							
Cajamarca							
Cerro de Pasco							
Chimbote							
Chincha							
Cusco							
Huancayo							
Huanuco							
Huaraz							
Ica							
Ilo							
Iquitos							
Juliaca							
Piura							
Pucallpa							
Tacna							
Talara							
Tarapoto							
Tumbes							
Intervention cities							
Control cities							
Overall							

Selected GPS Behavioral data - 2006

Gender	Ever had sex	Would use pharmacy for abnormal secretion	Condom use with last ptr	Sex with FSW	Condom with FSW	Sex with male	Condom with male
Male							
Female							
Overall							

Female Sex Worker (FSW) Survey Process Outcomes

	Participants					Samples provided			
						Blood		Vaginal samples	
City	Total approached	Not eligible	Declined to participate	Agreed to participate	Participation rate*	n	%**	n	%**
Ayacucho									
Barranca									
Cajamarca									
Cerro de Pasco									
Chimbote									
Chincha									
Cusco									
Huancayo									
Huanuco									
Huaraz									
Ica									
Ilo									
Iquitos									
Juliaca									
Piura									
Pucallpa									
Tacna									
Talara									
Tarapoto									
Tumbes									
Intervention cities									
Control cities									
Total									

FSW Survey Demographic Data

City	Age		Can read or write (%)	Ever married or cohabitant (%)	Living w/ sex partner (%)	Have dependents (%)	Charged to last client	
	mean	(range)					mean	(range)
Ayacucho								
Barranca								
Cajamarca								
Cerro de Pasco								
Chimbote								
Chincha								
Cusco								
Huancayo								
Huanuco								
Huaraz								
Ica								
Ilo								
Iquitos								
Juliaca								
Piura								
Pucallpa								
Tacna								
Talara								
Tarapoto								
Tumbes								
Intervention cities								
Control cities								
Overall								

STI Rates (one table for GPS, one for FSW)

Arm	City[8]	N[1]	Ct[2]	Gc[3]	Syph[4]	HIV[5]	N(female)[1]	Trich[6]	BV[7]
C	Ayacucho								
I	Cajamarco								
C	Talara								
I	Chincha								
C	Huancayo								
I	Cusco								
C	Tarapoto								
I	Huanuco								
C	Tacna								
I	Ica								
C	Barranca								
I	Pasco								
C	Huaraz								
I	Pasco								
C	Chimbote								
I	Piura								
C	Iquitos								
I	Pucallpa								
C	Ilo								
I	Tumbes								
C	Control								
I	Intervention								
	Total								

Notes:
[1]N is approximate for any given outcome since there may have been a few insufficient, lost or damaged specimens.
[2]Chlamydia by PCR (Aptima)
[3]Gonorrhea by PCR (Aptima)
[4]Syphilis by RPR (> 1:8)
[5]HIV by Elisa x 2 with WB confirmation
[6]Trichomonas by culture (In-Pouch)
[7]BV by ?
[8]Cities are arranged in pairs

PREVEN Trial Data Analysis Plan

Title: PREVEN Community Randomized Trial – A hybrid HIV/STD intervention on a high-risk group of female sex workers (FSW) and on the lower-risk young adult general population.

Background and Overview:
The primary aim of this trial is to evaluate the impact of a hybrid STD/HIV prevention intervention on the prevalence of sexually transmitted infections (STIs) (including gonorrhea, chlamydia, trichomonas, syphilis and HIV) in female sex workers (FSW) and the young adult general population (GP). The study is designed as a prospective community randomized trial of 20 medium sized cities in Peru. The intervention was begun in 2003 and the primary outcome (STI prevalence) will be measured in two cross sectional surveys (one in a population of FSW and the other in the general population of young adults) in 2006 in each of the 20 Peruvian cities. The surveys will also provide us with estimates of the fraction of HIV cases that are diagnosed and treated in existing programs (Ministry of Health, Army, Navy, Police health services, Social Security system and other programs in Peru), the prevalence of HIV viremia, the prevalence of bacterial vaginosis (BV) and risk behaviors. In secondary analyses we will use this information to identify characteristics of bridging populations and determining the structure of sexual networks. This information will be directly useful for planning nationwide programs that integrate prevention and treatment.

Broad objective: To evaluate the impact of a hybrid STD/HIV preventive intervention CRT on the prevalence of sexually transmitted infections (STIs), gonorrhea, chlamydia, trichomonas, and syphilis, in a high-risk group of female sex workers (FSW) and in the lower risk young adult general population.

Specific aims:
Primary

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the combined prevalence of gonorrhea and/or chlamydia and/or syphilis (RPR $\geq$ 1:8) and/or trichomonas (in women) in the general population of young adults.

Secondary

- To evaluate the impact of a hybrid STD/HIV preventive intervention on the combined prevalence of gonorrhea and/or chlamydia and/or syphilis (RPR $\geq$ 1:8, TP•PA confirmed) and/or trichonomas in female sex workers.
- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalences of bacterial vaginosis and HIV in the general population of young adults.
- To evaluate the impact of a hybrid STD/HIV preventive intervention on the prevalences of bacterial vaginosis and HIV on female sex workers.
- To evaluate the impact of a hybrid STD/HIV prevention intervention on condom use in the GP and FSW
- To determine if the effectiveness of the intervention differs in GP individuals who prefer to use pharmacies for treatment of STI-related symptoms
- To determine if the effectiveness of the intervention differs in GP men who use FSW

Study Design:
Overview: The study is a community randomized trial of 20 medium sized cities in Peru. The cities were initially paired based on STI prevalence measured in a baseline (pre-intervention) survey. One member of each pair was then randomized to receive the intervention, which consists of two primary components: i) pharmacist education to strengthen syndromic management of STI, and ii) outreach to female sex workers (FSW) using a mobile team. The primary outcome (STI prevalence) will be measured in two cross

sectional surveys - one among FSW and the other in the general population of young adults - in each of the 20 Peruvian cities after the intervention has been in place for approximately 2 years.

Baseline Surveys: Baseline surveys of STI prevalence in the general population (18-29 year old men and women), FSW and their clients were conducted in 24 midsize (> 50,000) cities in Peru in 2002 and 2003. These surveys collected both behavioral information and biologic samples.

The general population survey (GPS) used year 2000 census data to randomly select households by cluster sampling (each cluster was an average of 40 households). A random sample of clusters was selected for each city. Then a census was conducted for each household in the selected clusters and a random sample of households with eligible members was selected. Within each selected household, one eligible individual (male or female, aged 18 - 29, living in the city for at least 6 months) was randomly selected.

The FSW/client surveys used time-venue sampling. In each city the study team mapped known locations of FSW. Each day of the week was then divided into x parts and a random sample of time/venues was selected. All (?) sex workers present at the chosen venue at the chosen time were invited to participate in the survey.

Randomization: From the 24 cities that took part in the 2002 general population and 2003 FSW and client surveys, twenty were selected to participate in the CRT. Matching was employed to reduce imbalances in the randomization. Matching was based primarily on total STI prevalence (chlamydia, trichomoniasis, syphilis, gonorrhea, HIV) in the baseline general population survey. Population size and location (coastal, highland, jungle) were used as secondary matching criteria. Ten cities (one member of each matched pair) were then randomized to receive the intervention. Table 2 shows the matched pairs.

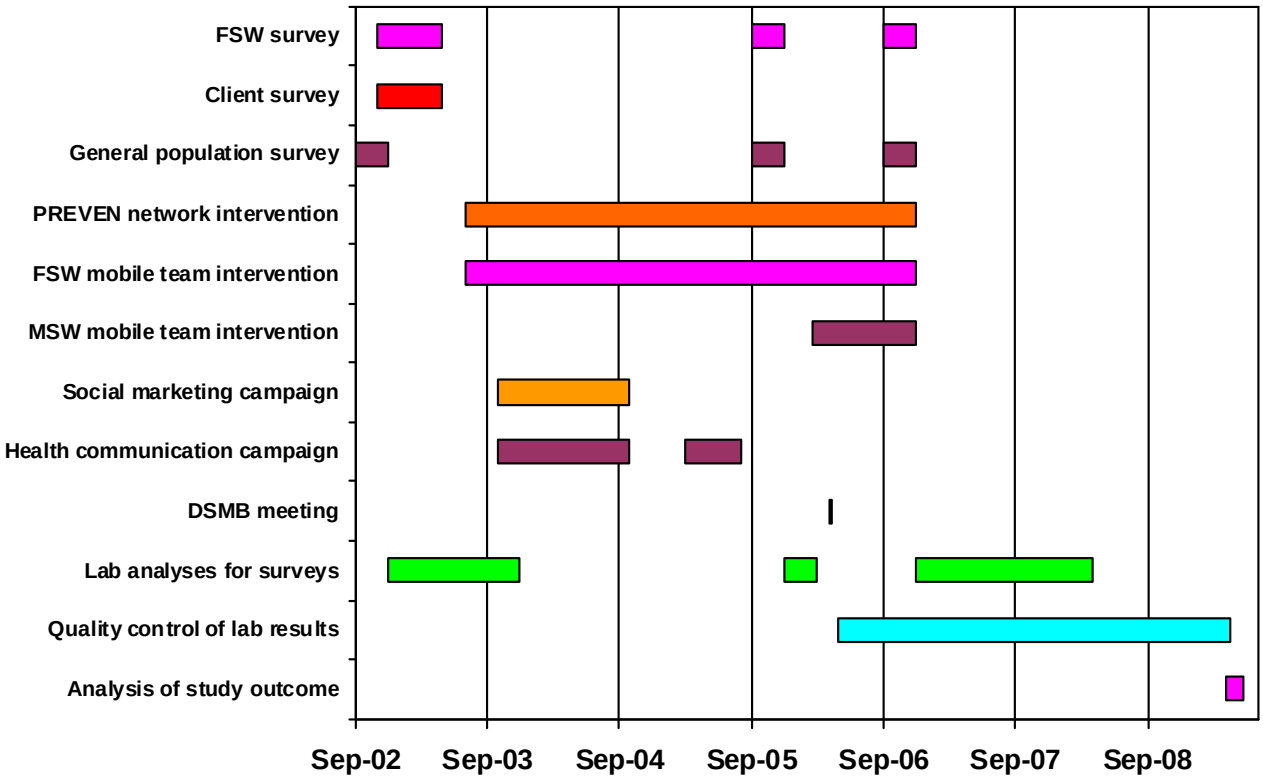
Table 2. Matched city pairs for the PREVEN project

	Matched pairs	
1	Chimbote	Piura
2	Ilo	Tumbes
3	Ayacucho	Cajamarca
4	Tacna	Ica
5	Huaraz	Cerro de Pasco
6	Huancayo	Cuzco
7	Talara	Chincha
8	Tarapoto	Huanuco
9	Iquitos	Pucallpa
10	Barranca	Juliaca

Intervention: The intervention consists of two key components: 1) training and support of pharmacy workers and referral networks of STD clinicians for improved STI recognition and management and for STD/HIV prevention counseling (RED PREVEN component); and 2) outreach to FSW via mobile teams (consisting of a health worker and a peer FSW educator) to increase STI screening and treatment, and condom use (FSW component). The intervention began in July 2003 and will continue through (at least) the final outcome surveys (December 2006). To supplement both intervention components, the study also included a one-year (October 2003 – October 2004) social marketing campaign of condoms with the provision of STD treatment packets and promotion of condoms' double protection against STDs/HIV and unwanted pregnancy.

Outcome Surveys: Interim surveys of the GP and FSW were conducted in 2005 and final outcome surveys will be conducted in 2006. The format and content of the surveys will be similar to the baseline surveys described above although the questionnaire data will be collected using a handheld (Palm) computer.

The figure below shows the timeline of the various components of the study.



Testable hypotheses: The testable hypotheses for this study will have the form

$$\text{Ho: } p_{control} = p_{intervention}$$
$$\text{Ha: } p_{control} \neq p_{intervention}$$

where p_i is the prevalence of the outcome (combined or individual STIs) in the i'th group. The section on inferential analysis below contains details on how this hypothesis will be tested.

Variables:

The following table lists variables that will be used in the statistical analyses.

Variable	**Definition**	**How collected**	**Units**	**Use in analysis**
pcrcturine	**CT in urine by PCR**	**GPS**	**0 = negative; 1 = positive; 2 = indeterminate**	**Outcome component**
pcrctswab	**CT in vaginal swab by PCR**	**GPS**	**0 / 1 / 2**	**Outcome component**
Ctresul	Prevalence of Chlamydia infection **by PCR**	Urine samples tested by Cobas (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5= indeterminate) in 2006, with description: "chlamydia trachomatis cobas result". Indeterminates treated as missing.** **GPS: Created variable for 2006 such that: vaginal swab results used where swab provided; otherwise urine result used. For men all samples were urine. Indeterminate results treated as missing.**	0 - 1	Outcome
Pcrgcurine	**GC in urine by PCR**	**GPS**	**0 / 1 / 2 (2= indeterminate)**	**Outcome component**
Pcrgcswab	**GC in vag. swab by PCR**	**GPS**	**0 / 1 / 2**	**Outcome component**
Genprgcuri	**GC in urine by PCR (Genprobe)**	**GPS**	**0 / 1 / 2**	**Outcome component**
Genprgcswa	**GC in swab by PCR (Genprobe)**	**GPS**	**0 / 1 / 2**	**Outcome component**
taqmangcur	**Taqman GC inurine**	**GPS - received variable in 2006 but contained no data – will we use this?**	**?**	**Didn't use**
taqmangcsw	**Taqman GC in swab**	**"**	**?**	**Didn't use**
Ngresul	Prevalence of gonorrhea infection **by PCR**	Urine samples tested by Cobas (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5=**	0 - 1	Outcome

		indeterminate) in 2006 with description: "neisseria gonorrhoea cobas result confirmed by aptima". Indeterminates treated as missing. **GPS: Created variable from other variables received in 2006 so that: Vaginal swab results used where women provided swab; otherwise result from urine sample used. For men urines used. We required positives to be confirmed by Genprobe. Indeterminates and positives without available confirmation results were treated as missing.**		
NewSyph	Prevalence of **elevated serum titer indicating recent** syphilis infection	Blood samples tested by RPR **and confirmed by TPPA**; result of each test is positive (titer $\geq$ 1:8) or negative (titer < 1:8) and individual results are aggregated to the city level***.** **GPS and FSW: Created variable in 2006 to accomplish this, using rpr, rpr dilution and tppa results.**	0 - 1	Outcome
HIVresul	Prevalence of HIV infection	Blood sample tested by dual ELISA and confirmed by WB; result of each test is positive or negative and individual results are aggregated to the city level* **FSW: variable arrived as 0/1/5 (5= indeterminate) in 2006 with description: "hiv result". Indeterminates treated as missing.** **GPS: created variable to match above description, from separate wb and elisa results.**	0 - 1	Outcome
Tvresul	Prevalence of trichomonas infection	Urine samples tested by InPouch culture (baseline) or Aptima (2006); result of each test is positive or negative and individual results are aggregated to the city level* (female only)	0 - 1	Outcome
BV	Prevalence of bacterial vaginosis	??; result of each test is positive or negative and individual results are aggregated to the city level* (female only)	0 - 1	Outcome
CtGcSy	Prevalence of	**GPS and FSW:** Created from	0 - 1	Primary

	Chlamydia and/or gonorrhea and/or syphilis infection	ctresult, gcresul, newsyph and **aggregated to** city level* **If any of the 3 test results was missing, combined outcome treated as missing.**		outcome
City	City code	assigned	1 - 20	
Arm	Randomization arm	assigned	0 = control 1 = intervention	Primary predictor
Pair	Randomization pair	assigned	1 - 10	Stratification variable
Age	Age (yrs)	GPS FF Q1.2		Covariate
Gender	Gender	GPS FF Q1.1	1=male 2=female	Covariate
Read	Can you read?	GPS FF Q1.4	1=yes 2=no 9=no response	
Married/Cohab	Marital status	GPS FF Q7.1	1=Single 2=Married 3=Cohabiting 4=Separated 5=Divorced 6=Widow/er 9=No response	
Eversex	Ever had sex:	GPS SA Q1.11 (female) Q1.12 (male)	0=no/1=yes	
Sexfsw	Sex w/prostitute:	GPS SA Q2.6 (males only, who replied 'yes' to 'eversex'),	0=no/1=yes	
Sexfswnum	How many times sex w/prostitute in last 12 months:	GPS SA Q2.9 (males only, who replied 'yes' to 'sexfsw')	0=None; 1-10=actual #; 11=more than 10; 66=doesn't remember	
Sexfswnocondom	How many times sex w/ prostitute w/o condom in last 12 months	GPS SA Q2.10 (males who gave response of 1 or more to 'sexfswnocondom');	1-4=actual #; 5=more than 4; 6=Always uses condom; 66=Doesn't remember	
Nocondc	Noncondom use w/ prostitute in last 12 months (men only):	Created variable Omission in code creating variable for interim analysis caused rates to be dramatically overestimated; See note.	1=yes (had sex with prostitute without condom in last year); 0=no (always used condom or no sex with prostitute)	Outcome

Sexmsm	Sex with male	GPS SA Q2.11 (males only)		
Msmnum	How many times sex w/male	GPS SA Q2.14 (males only, but variable "msmnum" received at interim analysis shows everyone responding to this including women and men who did not acknowledge MSM. Looks like not correct data for this question.)	0=None 1-4=? 66=Doesn't remember	
Msmnocond	How many times sex w/male w/o condom	GPS SA Q2.15 (males only, and those who acknowledge MSM.)	1-4=actual #; 5= more than 4; 6=Always uses condom; 66=Doesn't remember	
Nocondm	Noncondom use with male in last 12 months (men only)	Created variable Omission in code creating variable for interim analysis was an oversight and led to overstating rates; see note.	1=yes (had sex with male without condom in last year); 0=no (always used condom or no sex with male)	Outcome
Sexlastpartnum	Number of times sex with last partner in last 3 months	GPS SA Q3.11	1-20=actual # 21=more than 20; 66=doesn't remember	
Sexlastpartnocondom	Number of times sex with last partner in last 3 months without condom	GPS SA Q3.12	1-20=actual # 21=more than 20; 22=always uses condom; 66=doesn't remember	
Nocondl	Noncondom use w/last partner (GPS)	Created variable	1=yes (had sex with last sex partner without condom in last year); 0=no (always used condom or no sex in last 3 months)	Outcome
hlthseek	Health seeking behavior – "If you had abnormal secretion … what would you do first?"	GPS SA Q2.1	=Nothing =Consult friend =Pharmacy =Doc/Clinic =Hospital =Meds @ home =Other	
Workplace	Workplace type for	FSW questionnaire Q503 (last 7 days)	1=Brothel;	covariate

	FSW	or Q504 (last month)	2=Nightclub; 3=Bar; 4=Street; Other 99=no response	
Culinter	Condom use w/ last intercourse (FSW)	FSW questionnaire Q618 (is this only among women whose last sex was vaginal intercourse? – Q617?)	1=Yes 2=No (I created 'cl', same variable but 0=no/1=yes)	Outcome
	Can read/write?	FSW questionnaire – Q204	1=yes to both 2=can read 3=can write 4=neither	covariate
	Ever married or cohabitant?	FSW questionnaire – Q304	1=yes, 2=no, 99=no response	covariate
	Living with sex partner?	FSW questionnaire – Q309. For FSW, Will make 0/1 variable (1,2,4=yes, 3 or 5=no)	1=married live w/spouse; 2=married live with other sex ptr 3=married not living with sex ptr 4=not married, cohab; 5 = not married not cohab 99=no response	covariate
	Have dependents?	FSW questionnaire – Q310	1=yes, 2=no, 99=no response	Covariate
	Charged last client	FSW questionnaire – Q616 if last sex partner was client. If second to last sex partner was last client, then Q629. If sex partner before that, then Q643		

* To aggregate results to the city level the number of positive results in a given city/survey are divided by the number of nonmissing results from that city/survey.

Statistical Analysis:

Descriptive analyses

The following tables will be presented:

- Baseline general population survey (GPS) process outcomes by city (refusal rate, sample size, % giving blood, urine, swabs)[Cesar has done]
- Baseline STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) in the GPS by city [Cesar has done]

- 2006 GPS process outcomes by city (refusal rate, sample size, % giving blood, urine, swabs) [Cesar]
- 2006 GPS demographics (age, gender, etc) by city [UW]
- 2006 GPS STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) by city [UW]
- 2006 GPS rates of condom use among men with commercial sex partners in the last year, rates of condom use among men with male sex partners in the last year; rates of condom use among men and women with their last partner in the last 3 months; by gender [UW]

- Baseline female sex worker (FSW) survey process outcomes by city (refusal rate, sample size, % giving specimens) [Pablo has done]
- Baseline STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) in the FSW survey by city [Pablo has done]
- 2006 FSW survey process outcomes by city (refusal rate, sample size, % giving specimens) [Pablo]
- 2006 FSW demographics (age, workplace type, etc) by city [UW]
- 2006 FSW survey STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) by city [UW]
- 2006 FSW rates of condom use at the last sexual encounter; by city; by intervention arm [UW]

The following graph will also be presented:

- FSW STI rates (Ct, Gc, Syphilis, HIV, Trich, BV) over time in the interventions cities, pooled [Pablo]

Inferential analyses

Primary analysis

The unit of analysis will be the city (i.e. STI prevalence at the city level). This approach is appropriate since the sample size is approximately the same from city to city. The primary analysis of intervention efficacy will be based on the model

$$d_i^O = b_0 + b_1 d_i^B + \varepsilon_i \qquad \text{(M1)}$$

where d_i^O is the difference in the primary STI endpoint (combined gonorrhea, chlamydia, trich in women, and syphilis prevalence) between the intervention and control communities (control – intervention) of the *i*'th pair in the final (2006) outcome survey, d_i^B is the corresponding difference in the baseline survey and ε_i is a random error. The hypothesis of interest is

Ho: $b_0 = 0$	(no intervention effect)	(H1)
Ha: $\beta_0 \neq 0$	(intervention effect)	

Note that if d_i^B was not included in the model, this analysis would be equivalent to a paired t-test. The d_i^B term is included in the model to control for differences in STI prevalence at baseline (which may occur in spite of the matching).

Least squares regression will be used for model fitting. The standardized test statistic $\hat{b}_0 / se(\hat{b}_0)$ from this analysis has a t-distribution with 8 degrees of freedom. A p-value will be reported for the hypothesis (H1) and a type I error rate (α) of 0.05 (two-tailed) will be used to reject/fail to reject the null hypothesis (H1).

The endpoint in the primary analysis will be **combsti** as measured in the GP.

Secondary analysis

The analysis outlined above will also be used for the following endpoints:

- **combsti** in FSW
- **Ctresul, gcresul, newsyph, tvresul, HIVresul, BV** in GPS
- **Ctresul, gcresul, newsyph, tvresul, HIVresul, BV** in FSW
- **Nocondl** in GPS
- **Culinter** in FSW

To determine if the intervention is more effective in particular subsets of individuals (i.e. those who prefer to use pharmacies for treatment of STI-related symptoms) we will use an individual level analysis of the 2006 GPS data based on the following model

$$\text{logit}(p_{ij}) = b_0 + b_1 T_i + b_2 B_i + b_3 X_{ij} + b_4 T_i X_{ij} + a_i$$

where T_i is a 0/1 treatment indicator for city i, B_i is the baseline disease prevalence for city i, X_{ij} is a 0/1 covariate indicating whether subject j in city i belongs to the subset of interest (i.e. uses FSW) and a_i is a random city effect ($a_i \sim N(0,\sigma^2)$). The outcome in this analysis will be **combsti.** In the absence of the interaction term, the b_1 coefficient from this model estimates the overall treatment effect (comparable to b_0 in model H1). With the interaction term in the model, the hypothesis Ho: $b_4 = 0$ will be used to test if the intervention effect on combsti differs across levels of X (e.g. we might expect b_4 to be positive – intervention more effective – in individuals who prefer to use pharmacies for treatment of STI-related symptoms). A two-sided 0.05 level test will be used.

- To determine if the effectiveness of the intervention differs in individuals who prefer to use pharmacies for STI-related symptoms, X_{ij} = **hlthseek**.
- To determine if the effectiveness of the intervention differs in men who use FSW, X_{ij} = **sexfsw** and the analysis is restricted to men